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**THE ECOLOGY OF THE SANDFLY *LUTZOMYIA LONGIPALPIS*
IN AMAZONIAN BRAZIL**

A thesis submitted for the degree of Doctor of Philosophy (Ph.D.)
of the University of London

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BY

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ABSTRACT

In pursuit of effective control strategies against *Leishmania chagasi* and American Visceral Leishmaniasis, we investigated the ecology of *Lutzomyia longipalpis* in a series of laboratory and field experiments in Amazonian Brazil.

In Chapter II, we show that bloodfeeding success in peridomestic animal pens was dependent on the density of females feeding at the host. As the density of biting flies increased, hosts became more agitated, and bloodfeeding was interrupted. However, flies did not appear to distribute themselves between the available peridomestic hosts to minimise these costs.

In Chapter III, we infer from the results of mark-recapture experiments that pheromone-mediated attraction and arrest was the principal determinant of fly abundance in sheds. Males are also found to aggregate preferentially to the site of the previous night's activity. We use these results to explain the sub-optimal distribution of Chapter II.

In Chapter IV, we find that residual insecticide spraying caused a dramatic decrease in fly abundance in animal pens only when neighbouring aggregation sites remained untreated. Bringing together evidence from changes in *Lu.longipalpis* sex ratio, abundance of the different female *Lu.longipalpis* gonostates and abundance of other phlebotomine species, we argue that by disrupting pheromone production, the principal effect of spraying in this study was to stimulate the formation of aggregations at untreated and previously under-exploited sites, such as dining-huts, rather than the required mass-killing.

Finally, in Chapter V we report on laboratory feeding experiments that provide some evidence that digestion-mediated killing of parasites in the sandfly gut may be common to many types of animal blood.

In conclusion, we suggest that unless blanket spraying achieves close to 100% coverage, in the absence of a synthetic pheromone bait the best approach to disease control would be the selective treatment of susceptible host sites.

TABLE OF CONTENTS

TITLE PAGE	1
ABSTRACT	2
TABLE OF CONTENTS	3
LIST OF TABLES	8
LIST OF FIGURES	11
ACKNOWLEDGEMENTS	13
 CHAPTER I: GENERAL INTRODUCTION.	 15
<i>American Visceral Leishmaniasis</i>	16
<i>Lutzomyia longipalpis, Vector of American Visceral Leishmaniasis</i>	18
<i>Pre-imaginal ecology</i>	19
<i>Adult ecology</i>	20
<i>The Control of American Visceral Leishmaniasis</i>	22
<i>The Present Study</i>	23
 CHAPTER II: DENSITY-DEPENDENT FEEDING SUCCESS IN A FIELD	 26
POPULATION OF THE SANDFLY LUTZOMYIA LONGIPALPIS.	
 SUMMARY	 27
INTRODUCTION	28
MATERIALS & METHODS	30
<i>Study Area and Timing</i>	30
<i>Study Design</i>	31
<i>Classification of Catches</i>	32
<i>Morphometric Analysis</i>	33
<i>Regression Analysis</i>	33
RESULTS	36
<i>Sandfly Abundance</i>	36
<i>Determinants of Feeding Success</i>	38

<i>Dry season</i>	38
<i>Wet season</i>	43
<i>Bloodmeal and Fly Size</i>	45
DISCUSSION	48
<i>Determinants of Density-Dependent Feeding Success</i>	48
<i>Mechanism of Density-Dependence</i>	49
<i>Direct female-female competition</i>	49
<i>Host-mediated female-female competition</i>	49
<i>Costs of Density-Dependence</i>	50
<i>Reduced fecundity per gonotrophic cycle</i>	50
<i>Increased mortality per gonotrophic cycle</i>	51
<i>The Ideal Free Distribution</i>	52
<i>Between sheds within night</i>	52
<i>Within sheds</i>	53
<i>Sylvatic Versus Domestic Environments</i>	56
 CHAPTER III: PHEROMONES, KAIROMONES AND AGGREGATION	 58
BEHAVIOUR IN A FIELD POPULATION OF THE SANDFLY	
LUTZOMYIA LONGIPALPIS.	
 SUMMARY	 59
INTRODUCTION	60
MATERIALS & METHODS	62
<i>Study Area and Timing</i>	62
<i>Identification of Sandflies</i>	62
<i>Self-Marking Technique</i>	62
<i>Experiment 1: Aggregation at Male and Female Baits</i>	63
<i>Experiment 2: Aggregation Dynamics Within Nights</i>	63
<i>Migration to and from one shed</i>	64
<i>Migration between two sheds</i>	66
<i>Experiment 3: Aggregation Dynamics Between Nights</i>	66
RESULTS	68

<i>Experiment 1: Aggregation at Male and Female Baits</i>	68
<i>Experiments 2 & 3: Sandfly and Host Abundance</i>	68
<i>Experiments 2 & 3: Sandfly Sex Ratio</i>	71
<i>Experiment 2: Aggregation Dynamics Within Nights</i>	71
<i>Migration to and from one shed</i>	71
<i>Migration between two sheds</i>	76
<i>Experiment 3: Aggregation Dynamics Between Nights</i>	78
DISCUSSION	81
<i>Aggregation Dynamics Within Nights</i>	81
<i>Aggregation Dynamics Between Nights</i>	83
<i>Pseudoreplication in the Analysis</i>	84
<i>Implications for the Control of Lutzomyia longipalpis</i>	84
 CHAPTER IV: A SHED-LEVEL INTERVENTION TRIAL FOR THE CONTROL OF PERIDOMESTIC POPULATIONS OF LUTZOMYIA LONGIPALPIS.	 86
SUMMARY	87
INTRODUCTION	88
<i>The Impact of DDT House-Spraying on Leishmaniasis</i>	88
<i>The Impact of Outdoor Spraying on Lutzomyia longipalpis</i>	89
<i>New Methods in Sandfly Control</i>	93
MATERIALS & METHODS	95
<i>Study Area and Timing</i>	95
<i>Insecticide Regime</i>	96
<i>Study Design</i>	96
<i>Entomological Measures</i>	97
<i>Target-Sheet Removal</i>	98
<i>Analysis of the Focal Intervention</i>	98
<i>Analysis of the Blanket Intervention</i>	99
RESULTS	102
<i>Focal Intervention</i>	102
<i>Pre-treatment data</i>	102

<i>Chicken sheds</i>	102
<i>Dining-huts</i>	107
<i>Houses</i>	107
<i>Gonotrophic state</i>	110
<i>Mortality rate</i>	110
<i>Sex ratio</i>	114
<i>Blanket Intervention</i>	114
<i>Pre-treatment data</i>	114
<i>Chicken sheds</i>	116
<i>Dining-huts</i>	116
<i>Houses</i>	116
<i>Sex ratio</i>	119
DISCUSSION	120
<i>Focal Intervention</i>	120
<i>Blanket Intervention</i>	122
<i>Implications for the Control of Lutzomyia longipalpis</i>	124
 CHAPTER V: THE EFFECT OF BLOODMEALS ON PARASITE BURDENS IN LUTZOMYIA LONGIPALPIS.	 125
 SUMMARY	 126
INTRODUCTION	127
MATERIALS & METHODS	129
<i>Sandfly Stocks</i>	129
<i>Chick and Hamster Feeding</i>	129
<i>Membrane Feeding</i>	129
<i>Parasite Stocks</i>	130
<i>Breeding Conditions</i>	130
<i>Sandfly Dissection</i>	131
<i>Experiment 1</i>	131
<i>Experiment 2</i>	132
<i>Experiment 3</i>	132

<i>Experiment 4</i>	133
RESULTS	134
<i>Experiment 1</i>	134
<i>Experiment 2</i>	134
<i>Experiment 3</i>	137
<i>Experiment 4</i>	140
DISCUSSION	142
 CHAPTER VI: GENERAL DISCUSSION.	 145
<i>The Semiochemical Basis of Lutzomyia longipalpis Dynamics</i>	146
<i>Consequences for Vector Control</i>	147
<i>Future Work</i>	149
<i>Vector control</i>	149
<i>AVL epidemiology</i>	150
<i>Lutzomyia longipalpis population ecology</i>	151
 REFERENCES	 153

LIST OF TABLES

II.1	Proportions of females by gonotrophic status, from all traps from dry and wet season.	38
II.2	Parameter estimates from the minimum adequate model for the dry season data (Eq. II.6), and with only the night factor (D) and female abundance (N) (Eq. II.7).	40
II.3	Parameter estimates from the minimum adequate model for the wet season data (Eq. II.9).	44
III.1	Number of males and females caught at experimental chicken sheds with and without baits of 120 male or female <i>Lu.longipalpis</i> .	70
III.2	Regression estimates of the slope of the change in geometric mean unmarked and marked flies, and their ratio (unmarked:marked) between trap catches.	72
III.3	Correlates of the slope of the change in log marked flies (emigration rate) and the ratio of unmarked:marked (immigration rate) flies in individual sheds between trap catches 1-3 (2100-2400 hours).	75
III.4	Correlates of the log total number of marked males and females migrating to the 'away' shed caught in traps 1-4 (2100-0630 hours) with abundance of males, females and hosts in the home (h) and away (a) sheds.	77

III.5	The geometric mean proportion of flies caught in home sheds (h) that are marked (C) and the geometric mean proportion of marked flies that are caught at away sheds (a) on nights 1 (N1) and 2 (N2), and the geometric mean proportion of marked flies at the home shed that are caught on night 2.	80
IV.1	The proportion of females trapped in sheds, dining-huts and houses during the pre-treatment period in the four gonotrophic states: unfed, new fed, old fed and gravid.	104
IV.2	Genus, sub-genus, species and number of male phlebotominae, other than <i>Lutzomyia longipalpis</i> , caught in CDC traps from all locations over trapping rounds 1-8.	105
IV.3	Changes in the ln Odds Ratio (treatment:control) of <i>Lu.longipalpis</i> abundance in sheds for target and spray sheds.	106
IV.4	Changes in the ln Odds Ratio (treatment:control) of <i>Lu.longipalpis</i> from the dining-huts of target and spray shed homesteads.	108
IV.5	Changes in the ln Odds Ratio (treatment:control) of <i>Lu.longipalpis</i> from the houses of target and spray shed homesteads.	109
IV.6	Changes in the ln Odds Ratio of mortality (live:dead) of <i>Lu.longipalpis</i> in CDC traps from target and spray sheds.	113
IV.7	Changes in the ln Odds Ratio (treatment:control) of <i>Lu.longipalpis</i> abundance in sheds from the blanket intervention (* p<0.05; ** p<0.01).	118

V.1	Experiment 1. Numbers of <i>Lu.longipalpis</i> with parasite burden and amount of bloodmeal remaining in the gut, with or without a second meal of chick or hamster blood. ++ = much blood remaining in the midgut; + = little blood remaining; - = no blood remaining. N/A = Not Applicable.	135
V.2	Experiment 2. Number of <i>Lu.longipalpis</i> with parasite burden and amount of bloodmeal remaining in the gut, for flies fed parasites in <i>un</i> -heat-inactivated fox or chick blood.	136
V.3	Number (column percentage) of <i>Lu.longipalpis</i> surviving 5 days post-feed with parasites plus fox or chick blood.	137
V.4A	Experiment 3. Number of <i>Lu.longipalpis</i> with parasite burden and amount of bloodmeal remaining in the gut, for flies fed parasites in heat-inactivated fox or chick blood. First repeat.	138
V.4B	Experiment 3. Number of <i>Lu.longipalpis</i> with parasite burden and amount of bloodmeal remaining in the gut, for flies fed parasites in heat-inactivated fox or chick blood. Second repeat.	139
V.5	Experiment 4. Change in parasite number <i>in vitro</i> , in four classes of blood, over time. HF: heat-inactivated fox blood; HC: heat-inactivated chick blood; UF: un-heat-inactivated fox blood; UC: un-heat-inactivated chick blood.	141

LIST OF FIGURES

II.1	Frequency distribution of male (\\) and female (///) <i>Lu.longipalpis</i> and female mosquito (\equiv) abundance from wet and dry season trap catches.	37
II.2A	Proportion of the total female <i>Lu.longipalpis</i> with a new bloodmeal in dry season trap catches versus ln total female abundance in traps.	41
II.2B	Proportion of female <i>Lu.longipalpis</i> in dry season trap catches with new bloodmeals that are large versus ln total female abundance in traps; observed (Δ) and fitted values (\square) from the minimum adequate model (Eq. II.6).	42
II.3	Frequency distribution of bloodmeal sizes (measured in plan) of all bloodfed female <i>Lu.longipalpis</i> from a random sample of trap catches from the dry and wet season; flies classified as small meals (\\) and large meals (///) on the day of capture.	47
II.4	The change in feeding success, G , within shed, with a constant female density, N , and increasing host density, Q , calculated from the minimal model (Eq. II.6) with three values of v : (a) $v = 0$; (b) $v = -m$; (c) $v = 0.388$.	55
III.1	Total catch (males + females) from 2100-0630 hours versus host abundance for each shed-night in experiment one. The line represents the fitted values from regression with the quadratic term host^2 .	69
III.2	Changes in log abundance of marked (\square) and unmarked (Δ) flies and their ratio (marked:unmarked) (\circ) in sheds over traps 1-4 (2100-0630 hours). A = males; B = females.	73

IV.1	Changes over time in the abundance of <i>Lu.longipalpis</i> (males+females) in control sheds (x), sheds with target-sheets (+), sprayed sheds (Δ) and VF sheds (\square), and daily mean precipitation (filled bars). Empty bars = data not available.	101
IV.2	Changes over time in the abundance of <i>Lu.longipalpis</i> (solid line) and other phlebotomines (broken line) at spray homesteads (Δ) and target-sheet homesteads (+), relative to control homesteads (set to 1). Shed = a; dining-hut = b; house = c.	103
IV.3	Changes over time in the abundance of unfed (\blacksquare), new fed (+), old fed (x) and gravid female <i>Lu.longipalpis</i> (Δ) caught in treated sheds, relative to control sheds (set to 1).	111
IV.4	Changes over time in the ratio of live:dead <i>Lu.longipalpis</i> (solid line) and other phlebotomines (broken line) in sprayed sheds (Δ) and sheds with target-sheets (+), relative to control sheds (set to 1). Ratio of live:dead <i>Lu.longipalpis</i> in sheds with target-sheets relative to the same sheds with target-sheets removed (\square).	112
IV.5	Changes over time in the sex ratio (males:females) of <i>Lu.longipalpis</i> in sprayed sheds (Δ), sheds with target-sheets (+) and VF sheds (\square), relative to control sheds (set to 1).	115
IV.6	Changes over time in the abundance of <i>Lu.longipalpis</i> (solid line) and other phlebotomines (broken line) at VF homesteads (Δ), relative to control homesteads (set to 1). Shed = a; dining-hut = b; house = c.	117

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SABOR AÇAÍ

É para que tu foi plantado,
É para que tu foi plantada,
Para invadir a nossa mesa,
E abastar a nossa casa.

Teu destino foi traçado,
Pelas mãos da mãe do mato,
Mãos prendados de uma deusa,
Mãos de toque abençoado.

És a planta que alimenta,
A paixão do nosso povo,
Macho, fêmea das touceiras,
Onde Oxossi faz seu posto.

A mais magra das palmeiras,
Mas mulher do sangue grosso,
E homen do sangue vasto,
Tu te entrega até o caroço.

E tua fruta vai rolando,
Para os nossos alguidares,
E se entrega ao sacrifício,
Fruta santa, fruta mátir,
Tens o dom de seres muito,
Onde muitos não tem nada,
Ums te chamam açaizeiro,
Outros te chamam jasara.

Põe tapioca, põe farinha d'agua,
Põe açúcar, não põe nada,
Ou me bebe como um suco,
Que eu sou muito mais que um fruto,
Sou o sabor Marajoara, sou o sabor Marajoara, sou o sabor...

Nilson Chaves

CHAPTER I

GENERAL INTRODUCTION

American Visceral Leishmaniasis

Leishmania chagasi is the protozoan agent of American Visceral Leishmaniasis (AVL), which has been reported from 14 countries throughout the Americas, from Mexico to Argentina (Grimaldi *et al* 1989). The disease mainly affects children, particularly the malnourished (Badaró *et al* 1986, Cerf *et al* 1987, Dye & Williams 1993), and other immuno-compromised groups (e.g. Badaró *et al* 1987, Gradoni *et al* 1993).

There are an estimated 1.6 million people at risk of infection with this potentially fatal disease, and 16 000 clinical cases annually (Ashford *et al* 1992). Although published prevalences are unreliable, there is little doubt that Brazil, within whose borders more than 90% of all cases have been reported, constitutes the major endemic focus of the disease (Grimaldi *et al* 1989). To date, all the reported cases from the Brazilian Amazon have come from the State of Pará. Here AVL has traditionally been regarded as rare and sporadic. In 1984, however, an epidemic of 51 new clinical cases was reported from Santarém, a large town about 800km inland on the Amazon river, prompting a reassessment of its importance in the region (Lainson *et al* 1985). Since then, it has been found to be endemic in various areas of Pará state, such as Igarapé Miri and Marajó Island. In our study area of Salvaterra District, on the eastern coast of Marajó Island, 16 cases were reported to the Instituto Evandro Chagas in Belém between 1993 and 1994. This is probably a considerable under-reporting of cases in an area which typically lacks qualified medical practitioners (F. Silveira, pers. comm.).

There has been some debate over the origin of *L.chagasi* (Lainson & Shaw 1976, Killick-Kendrick *et al* 1980, Lainson *et al* 1987) but it is now generally accepted that it is synonymous with *Leishmania infantum*, one of two Old World visceralising leishmaniases (Momen *et al* 1987, Beverley *et al* 1987, Rioux *et al* 1990). It is considered most likely that the parasite arrived

in the New World in infected dogs from Europe and perhaps West Africa (Killick-Kendrick *et al* 1980).

The crab-eating fox, *Cerdocyon thous*, is believed to be the sylvatic reservoir of *L.chagasi*, by which vehicle the parasite is disseminated from village to village (Lainson *et al* 1969, Silveira *et al* 1982, Lainson *et al* 1990). However, there is no evidence yet of a fox-fox cycle, and recent work suggests that foxes may not be sufficiently infectious for this rôle (O. Courtney, unpublished work). *L.chagasi* has also been reported from the opossum *Didelphis albiventris* in North-Eastern Brazil (Sherlock *et al* 1984), and the neotropical opossum, *Didelphis marsupialis* in Colombia (Corredor *et al* 1989, Travi *et al* 1994). However, these animals have not yet been shown to be a widespread host, and despite a large number of examinations, no parasites have been isolated from *D.marsupialis* in Pará (R. Lainson, unpublished work).

The major peridomestic reservoir of *L.chagasi* is therefore the dog, *Canis familiaris*, in which host the disease is referred to as Canine Visceral Leishmaniasis (CVL). In Salvaterra district, for example, disease prevalence exceeds 50% of dogs in rural areas (Courtney *et al* 1994), and dogs can be highly infectious (O. Courtney, unpublished work). From dogs the parasite is transmitted to humans via the insect vector. Humans are usually uninfected to the sandfly vector (Deane & Deane 1962). However, recent work has shown that in the case of HIV patients, transmission of *L. infantum* to the European vector *Phlebotomus perniciosus* is possible, raising the possibility that wholly anthroponotic cycles of disease transmission may be possible (Molina *et al* 1994).

Lutzomyia longipalpis: Vector of *Leishmania chagasi*

Adult phlebotomine sandflies of the species complex *Lutzomyia longipalpis* are the major vectors of AVL, occurring almost throughout the range of the parasite (Lainson & Shaw 1979). Recently, a second vector of the disease, *Lutzomyia evansi* has been incriminated in Córdoba, Colombia, an area outside the range of *Lu.longipalpis* (Travi *et al* 1990). However, although circumstantial evidence for its involvement in disease transmission has also been reported from Margarita in Venezuela, and Costa Rica, it is unlikely that *Lu.evansi* plays a wide rôle in disease transmission (reviewed in Travi *et al* 1990). Ryan *et al* (1984) also report catches of *Lutzomyia antunesi* with suspected leishmania infections on Marajó, but attempts to identify the parasite involved were not successful.

The existence of sibling species of *Lu.longipalpis* has been demonstrated from a number of areas in Latin America by mating experiments (Ward *et al* 1983a, Ward *et al* 1983b, Ward *et al* 1988) and genetic studies (Lanzaro *et al* 1993). Breeding compatibility between populations of *Lu.longipalpis* (s.l.) seems to be predicted in part by production of one of two classes of a pheromone produced from tergal glands on the male (Phillips 1986). More detailed analysis has shown an ever more complicated array of chemicals present in gland extracts, differing between populations from different regions (Hamilton & Ward 1991), yet such fine variations in composition have not yet been shown to constitute a mating barrier.

The site of the present study, on Marajó Island, Pará, in Northern Brazil, constitutes one of the best studied populations of *Lu.longipalpis* in the field (e.g. Lainson *et al* 1983; Lainson *et al* 1990; Dye *et al* 1991; Quinnell *et al* 1992; Quinnell & Dye 1994a,b). The fly was first identified from Marajó on February 1982, in conjunction with human cases of AVL (Lainson *et al* 1983), and further incriminated by Ryan *et al* (1984). Dye *et al* (1991) suggest that only

one member of the species complex is to be found within our study area. However, this is based on phenotypic characters, and awaits the more reliable analysis of pheromone profiles or mating experiments.

Pre-Imaginal Ecology. Almost nothing is known about the larval ecology of sandflies in general (for reviews see: Hanson 1961; Bettini 1987; Killick-Kendrick 1987), and *Lu.longipalpis* is no exception. An early study in Pará State recovered no *Lu.longipalpis* larvae despite extensive searches around the homestead (Castro-Ferreira *et al* 1938). Deane & Deane (1957), working in Ceará State, Brazil, found a total of 19 larvae from four locations: a mule corral (12), under a rock in sunlight (3), under a rock in shade (1) and in a fissure on a rock promontory (3). Bettini & Melis (1987), working with Old World phlebotomines, summed up our current understanding of the larval micro-habitat when they wrote that larvae are associated with "*a relatively stable, cool and humid environment, protected from sunshine and rain, rich in... organic nitrogen*", a definition which has progressed little since the pioneering work of Howlett (1913).

Not surprisingly, there has been no field research into *Lu.longipalpis* larval ecology. In the laboratory, however, a series of experiments have established the existence of an oviposition pheromone secreted onto eggs by the accessory glands of *Lu.longipalpis* (El-naïem & Ward 1990; El-naïem *et al* 1991; El-naïem & Ward 1991; Dougherty *et al* 1992). The range over which the pheromone operates is not known, and it is therefore unclear whether it acts as an attractant or short-range oviposition stimulant. El-naïem & Ward (1992a) have also shown that various organic substances - colony debris, larval food and rabbit faeces - also stimulated oviposition. This is consistent with our own results obtained with leaf fragments in experiments conducted at the Instituto Evandro Chagas, Belém, Brazil (data not shown). It is also known that

Lu.longipalpis demonstrates thigmotropic oviposition behaviour, laying its eggs preferentially in crevices rather than flat surfaces (Elnaiem & Ward 1992b).

The evidence to date is equivocal on the subject of how breeding sites might be distributed: the few field catches made have been from diverse, dispersed sites; studies of oviposition pheromone suggest that sites should be aggregated, but the scale of aggregations is not predicted. This is not merely of academic interest. If sites were highly aggregated, they would be more readily controllable, assuming they could be reliably located.

Adult Ecology. Male and female *Lu.longipalpis* are active at night between approximately 1800 hours and 0600 hours (Quinnell & Dye 1994a). Both sexes take sugar meals, but in common with many other blood-sucking diptera, only the female takes a bloodmeal. This it is thought to do only once per gonotrophic cycle - the cycle of blood-feeding, mating and oviposition - taking a single, full meal, which it requires for egg maturation (Ready 1979). On Marajó, the mean duration of a gonotrophic cycle is 3 days (Dye *et al* 1991). *Lu.longipalpis* is a multivoltine species with numerous overlapping generations, and on Marajó adults may be found throughout the year. Seasonality in the abundance of *Lu.longipalpis* has been studied at a number of sites with differing climates. The evidence suggests that marked seasonality is the result of patterns of rainfall. In Ceará State, Brazil (Deane & Deane 1962), and El Callejon, Colombia (Morrison *et al* 1995), where annual rainfall is low, *Lu.longipalpis* abundance is positively correlated with rainfall. In Costa Rica, where rainfall is much higher, populations were at their greatest just before the rainy season (Zeledón *et al* 1984). It is suggested that precipitation affects the availability of breeding sites (Morrison *et al* 1995), implying that there is an ecological window within which moisture in the breeding site is neither too high nor too low, and which is satisfied at different times in the season depending on the

climate of the region.

Although *Lu.longipalpis* is believed to be sylvatic in origin (Lainson *et al* 1990), the species thrives in the peridomestic environment. At dusk, large aggregations of males and females assemble on or near hosts, where bloodfeeding and mating occur. The major foci of this activity are animal pens, and in particular chicken sheds, where thousands may be caught in a single CDC light-suction trap, ten-fold more than in houses (Quinnell & Dye 1994b). By dawn, however, most flies have exited the shed for unknown resting sites (Quinnell & Dye 1994a).

Females are catholic in their feeding habits, seeming to discriminate between hosts -at least in the case of birds and mammals - on the basis of size only (Quinnell *et al* 1992), and the difference in abundance between houses and animal pens is principally the result of host accessibility (Quinnell & Dye 1994a). Host location is assisted by host kairomones and male *Lu.longipalpis*, which produce a powerful pheromone capable of attracting females and other males over several meters (see Chapter III for a review of the aggregative response of *Lu.longipalpis* to semiochemicals). Short-range communication includes intensive wing fluttering by both sexes to produce acoustic signals during aggression between males, courtship and mating (Ward *et al* 1983a). Like the male pheromone, this varies between sibling species, which produce 'songs' of different inter- and intra-pulse frequency, and may help constitute a mating barrier.

The mechanism of sexual selection which underpins aggregation behaviour has been studied by Jarvis & Rutledge (1992). They provide evidence which goes some way towards satisfying the definition of lekking promulgated by Bradbury (1981): that males, defending territories containing no resource for the female save the males themselves, are freely chosen by females for the sake of their genetic material alone. This work has been considerably extended by Jones (1995), and preliminary results suggest that *Lu.longipalpis* is indeed a lekking species.

The pattern of AVL epidemiology in Latin America is changing. The incidence of disease is increasing in urban areas such as Fortaleza, Natal, Teresina, São Luís, Santarém, Belo Horizonte and Rio de Janeiro (*Lainson 1989, Costa et al 1990, Bezerra et al 1992, Michalik et al 1992, Jerônimo 1994, Marzochi et al 1994*). This has been attributed to the large-scale migration of people from rural to urban areas, accompanied by numerous domestic animals, and settling in poorly-constructed, high-density shanty towns (*Tesh 1995*). This constitutes an excellent habitat for *Lu. longipalpis* and transmission of *L. chagasi* between dogs and to humans. The inefficiency of current control practices has been further highlighted by the emergence of AVL in HIV patients from areas previously thought to be free from transmission (e.g. *Badaró et al 1987, Gradoni et al 1993*).

The debate over how best to interrupt parasite transmission to humans centres on the control of CVL, and has been the subject of recent reviews (*Dye et al 1994, Tesh 1995, Dye 1996, Dye et al 1996*). Brazilian control programmes typically take an integrated approach, combining identification and destruction or treatment of seropositive dogs with insecticide spraying in pursuit of mass-killing of vector populations (reviewed in Chapter IV). They are typically *ad hoc* responses to epidemics, and are poorly monitored. As a result, little is known of which (if any) of the control elements are important in disease control (see review in Chapter IV).

Dye et al (1994, 1996), developing a series of models of disease epidemiology, reach the conclusion that dog destruction is the least effective strategy, since canine surveillance programmes are labour intensive and expensive, owner compliance (for which there is no legal obligation) may be poor, and puppies are bought to replace destroyed animals and will quickly

become infected in their turn. A marker for infectiousness, rather than infection, would greatly reduce the number of dogs which would have to be destroyed, but has not yet been identified. Treatment, as opposed to destruction, avoids replacement with puppies, but is far more expensive and seems not to prevent recrudescence of the disease, particularly in symptomatic cases (Tesh 1995). Both Dye *et al* (1994, 1996) and Tesh (1995) agree that a putative canine vaccine would perform considerably better than treatment, but this option awaits development.

The alternative to targeting dogs is vector control. Dye (1996) presents evidence from epidemiological models to suggest that insecticide spraying would have a substantially greater impact on ZVL than a dog vaccine. Furthermore, although the breeding sites and adult resting sites are cryptic, the dense *Lu.longipalpis* mating/feeding aggregations in animal pens present an obvious target for residual insecticide programmes. However, as is inevitable with such models, the results of Dye (1996) are not free from assumptions about the ecology of the vector. This is sandfly control under the best circumstances, assuming that there are no features of *Lu.longipalpis* ecology which would frustrate attempts at mass-killing, or compensate for it.

In pursuit of a rational basis for the design and implementation of control strategies against the vector, we therefore investigate some aspects of the ecology of mating and bloodfeeding in *Lu.longipalpis* - the point in their life history at which they are most amenable to control - and their implications for disease epidemiology and control.

The Present Study

Density-dependent processes tend naturally to control population size by reducing the fitness of individuals in the population as density increases (Begon & Mortimer 1986). Such processes can also confound artificial attempts to control vector abundance by compensating for

increased mortality. Density-dependent bloodfeeding success has been reported for several species of bloodsucking insects (see review in Chapter II). Although this has never been reported for a sandfly, it seems reasonable to suppose that it might exist. If so, the success of attempts to reduce the abundance of sandflies would be inversely proportional to the intensity at which such a mechanism is operating. Given the densities at which *Lu.longipalpis* is found in the homestead, intensity seems likely to be high. We therefore begin by investigating non-linearities in the bloodfeeding success of *Lu.longipalpis* (Chapter II).

Behavioural traits which determine the way a vector distributes itself within its environment, and consequently how it reacts to changes in that environment, may also adversely affect the outcome of control measures. Repellency of flies from some insecticides provide an example of this. The major determinants of *Lu.longipalpis* distribution during bloodmeal- and mate-seeking are thought to be host kairomones and the male pheromone (reviewed in Chapter III). We therefore investigate, qualitatively and quantitatively, the effect of host and fly abundance on the dynamics of fly aggregations in chicken sheds through a series of mark-recapture experiments in Chapter III.

In Chapter IV, we test predictions arising from Chapters II & III about the effect of insecticide intervention on *Lu.longipalpis* abundance and distribution in a field trial with the synthetic pyrethroid lambda-cyhalothrin. Prompted by the success of insecticide-impregnated targets for the control of other sandfly species (reviewed in Chapter IV), we also test the efficacy of target cloths against traditional residual spraying techniques.

The effects of sandfly abundance and distribution on disease epidemiology may be confounded by any relationship between the size and type of bloodmeal. There is evidence from other leishmania-sandfly systems to suggest that digestion of new bloodmeals can cause infected flies to lose their parasites (reviewed in Chapter V). Results from Chapter II suggest that

Lu.longipalpis is often obliged to take the blood it requires for each gonotrophic cycle in a series of small meals over the course of more than one night, rather than as a single large meal. This raises the possibility of a large, and density-dependent effect on parasite transmission, as flies first become infected and then lose their meals within the same gonotrophic cycle, before becoming infectious. In Chapter V, we therefore investigate the effect of bloodmeal digestion on *L.chagasi* parasite burdens in *Lu.longipalpis*.

Finally, in Chapter VI we attempt a synthesis of the results and discuss the possible areas of future on vector control, vector ecology and disease epidemiology which this study suggests.

CHAPTER II

DENSITY-DEPENDENT FEEDING SUCCESS IN A FIELD POPULATION OF THE SANDFLY, *Lutzomyia longipalpis*

SUMMARY

- (1) A two-stage observational study of sandfly populations in chicken sheds was conducted on Marajó Island, Northern Brazil, to identify determinants of feeding success within populations of female *Lutzomyia longipalpis* during the dry and wet seasons.
- (2) We show, for the first time for a sandfly population, that *per capita* feeding success, measured as bloodmeal size, decreases with increasing density of other females at the feeding site, and increases with host density.
- (3) Interference with sandfly feeding is evidently host-mediated, as with some other bloodsucking insects.
- (4) The fact that female feeding success varies between sheds suggests that, with respect to bloodfeeding, female sandflies are not distributed according to the Ideal Free Distribution (IFD): i.e. they do not maximize individual resource gains. Probable costs of reduced bloodmeal size are discussed in terms of female fecundity and mortality.
- (5) By fitting a generalised version of Sutherland's interference model, which allows patch quality as well as female interference to vary non-linearly, we infer that bloodfeeding within sheds is predominantly on a subset of the available fowl. This too is consistent with the view that female flies disobey IFD.
- (6) We also demonstrate that increasing densities of female mosquitoes are associated with smaller bloodmeals in female *Lu.longipalpis*, suggesting that competition for bloodmeals can also occur between families of bloodsucking insects.

INTRODUCTION

Studies with mosquitoes (Webber & Edman 1972; Edman *et al* 1972; Kale, Edman & Webber 1972; Nelson *et al* 1976; Klowden & Lea 1979; and Waage & Nondo 1982), horseflies (Waage & Davies 1986), tsetse flies (Vale 1977) and reduviid bugs (Schofield 1982) have shown that an increasing density of these blood sucking insects on a host leads to increasing defensive behaviour by the host as the insects bite at greater frequency. Host defensive behaviour interrupts feeding flies, and this manifests itself as a reduced number of bloodmeals. Furthermore, the average size of those bloodmeals falls, resulting in fewer eggs. Thus density-dependent feeding success is very closely linked to fecundity and the genetic contribution that an individual makes to the next generation. However, the great majority of the studies mentioned above have been conducted in laboratory cage experiments (but see Waage & Davies 1986). It is therefore unclear whether host-mediated density-dependent feeding success operates commonly under natural conditions, and whether it is important for population regulation, a subject little understood for bloodsucking arthropods (Dye 1992).

As Kalmus and Hocking (1960) point out, the process of blood-feeding in nature is a series of events, of which probing and biting are only one stage. Before settling to probe and bite, for example, female mosquitoes must make a decision about how they should distribute themselves over the available resources. Ideal Free Distribution (IFD) theory predicts that on any given night, a population of flies should distribute themselves in such a way as to maximize feeding success, and therefore minimize the density dependent effects found in cage experiments.

Density-dependent feeding success has never been shown for a sandfly species, and rarely for any bloodsucking dipteran in the field. Yet if it does occur, it seems very likely that

the cost of a mating system with pheromone-driven aggregation of the order seen in peridomestic populations of *Lu.longipalpis* would be substantial. In this observational study of sandfly populations in chicken sheds in rural Amazonian Brazil, we therefore attempt to answer two questions: does density-dependent feeding success operate in natural populations of *Lu.longipalpis*, and do flies distribute themselves so as to minimise these costs?

MATERIALS AND METHODS

Study Area and Timing

The study took place in Salvaterra district (Latt. 0°45', Long. 48°30') on the island of Marajó, State of Pará, in northern Brazil. This is a rural zone, with between 10 and 25 inhabitants per square kilometre (Anon. 1981). The vegetation is predominantly cerrado (savanna), and secondary growth where *terra firme* forest has been felled for agricultural purposes. Seasonally flooded (*varzea*) forest is found following water courses, and villages are situated on forest borders. Villages were of 32-38 houses in size, arranged in a predominantly linear pattern along dirt roads. Homesteads were usually planted with fruiting trees, and domestic animals - dogs, pigs and fowl - were common.

Fowl were housed in sheds comprising a close palisade of wooden stakes and a roof of *najá* palm. Where there was no shed, fowl usually roosted in trees. The mean abundance of each type of fowl per shed (n=31) were: chickens: 8.42 (S.E. 1.01); ducks: 0.28 (S.E. 0.14); guinea fowl: 0.17 (S.E. 0.12); turkeys: 0.056 (S.E. 0.06).

An initial phase of trapping (dry season) began on October 16 1993, and ended on November 11 of the same year, towards the end of the dry season in Northern Brazil. Meteorological conditions in the area were relatively constant over this first sampling period, with an average temperature of 27.9°C (sd. 0.27)(@21.00hrs), relative humidity of 79.1% (sd. 2.79)(@21.00hrs) and daily precipitation of 0.1mm (sd. 0.29)(data from the National Institute of Meteorology, MAARA, Belém, Brazil).

A second phase of trapping (wet season) began on December 12 1993, at the beginning of the rainy season, and ended on February 22 1994. During this period, the meteorological

conditions changed markedly, and became more variable. The average temperature fell to 26.8°C (sd. 1.244)(@21.00hrs), mean relative humidity was 85.2% (sd. 6.79)(@21.00hrs) and mean daily precipitation was 13.8mm (sd. 26.42).

Study Design

The present study was part of a larger project investigating the effect of two insecticide treatments on sandfly populations in chicken sheds. In October and November of 1993 the pre-treatment (dry season) data from 31 sheds were collected in two trapping rounds of ten nights, three to four sheds per night (total 62 sheds). These sheds form the resource patches, i (Eq. II.1), between which females distribute themselves. Sheds on any one night were never more than fifty meters apart. This is considerably less than the maximum distances which *Lu.longipalpis* have been recorded travelling in a night. Therefore by trapping more than one shed per night, we were able to investigate the distribution of sandfly populations between sheds on any one night.

The wet season data was taken from traps in a sub-set of ten untreated (control) sheds during the post-treatment period, in four trapping rounds of ten nights, one shed per night (total 40 sheds). These catches were made in order to examine the interaction of sandflies with mosquitoes, which only assume significant densities after the rains begin.

In all cases, sampling was carried out with CDC light-suction traps placed in sheds from 18.00hrs to 06.00hrs, encompassing the entire period of sandfly activity in sheds (Quinnell & Dye 1994a). Data were excluded from the analysis if catches were not examined on the day of trapping, traps had become soaked by rainfall, trap batteries had weakened or stopped working or if hosts other than domestic fowl were also resident in the shed. Sheds were in four villages, the two furthest being approximately five kilometres apart.

Classification of Catches

Catches were aspirated from traps, mostly alive, on the morning of collection. *Lu.longipalpis* were separated by eye, and any doubtful specimens were mounted and identified by external morphology and/or spermathecal structure (Ryan 1986). Numbers of males and females were recorded, and females were sub-divided, on examination at x20, into:

- (a) Unfed - no blood meal, no ovarian development.
- (b) Small New Meal - bright red blood meal not sufficient to distend the abdomen. No ovarian development.
- (c) Large New Meal - bright red blood meal sufficient to distend the abdomen. No ovarian development.
- (d) Small Old Meal - black blood meal in median mid-gut not sufficient to distend the abdomen. No ovarian development.
- (e) Large Old Meal - black blood meal - often digested from the posterior mid-gut forward and associated with large, opaque ovaries - sufficient to distend the abdomen.
- (f) Gravid - no blood meal remnant. Ovarian development, with eggs usually visible as dark striations within the abdomen.

Dye *et al* (1991) found that the mean duration of a gonotrophic cycle was three days, and it is assumed here that 'new meals' correspond to day one, 'old meals' to day two, and gravid flies to day three post-feed. Division of blood meals into large or small gives a discontinuous but conveniently measured index of the mean size of meals taken in a shed. A continuous measure of blood meal size was not practical to take due to pressure of time and resources.

Mosquitoes were classified simply as male or female on the basis of antennal setae: plumose antennae were classified as male, sparsely-haired antennae as female (Kettle 1984). No attempt was made to identify specimens to genus or species level on a regular basis. Additionally each shed was censused on the occasion of each sample for number, type and size of fowl. An index of relative fowl abundance was constructed, *a priori*, by weighting for size - adults counted as 1, pullets as 0.5 - giving the estimate for patch quality, Q_i .

Morphometric Analysis

A sub-sample of newly-fed sandflies from the dry season catches was stored in alcohol and later mounted in Berlese "Gum Chloral" mounting medium (GBI Laboratories, Manchester, England). Using VIDEOPLAN software (Kontron Elektronik, Germany), wing length was measured, from the apex at vein R5 to the point where R5 splits to meet R2 and M2, as an index of body size. The area of the blood meal viewed in plan was also measured to provide a continuous index of bloodmeal size.

Regression Analysis

We can investigate the behavioural ecology of sandfly feeding success by adopting and extending theory developed to study the hypothesis that foraging animals follow the Ideal Free Distribution. The simplest form of IFD is the 'Continuous Input Model' under the assumption that all individuals are of equal competitive ability. Thus the number of resource items gained

by an individual is a function of the number of competitors and resources per patch:

$$G_i = Q_i/N_i \quad (\text{II.1})$$

where G_i , Q_i and N_i are resources gained, patch quality (ie resources available) and number of competitors respectively at patch i . To maximize the gains per individual, competitors must distribute themselves so that the ratio, Q/N is the same over all patches, known as the input matching rule (Parker 1978).

The continuous input model (Eq. II.1) assumes that competition between foraging organisms changes linearly with density. However, this is rarely the case, and this is recognised in a modification of equation (II.1) known as Sutherland's (1983) interference model:

$$G_i = Q_i/N_i^m \quad (\text{II.2})$$

or, linearly,

$$\ln G_i = \ln Q_i - m(\ln N_i) \quad (\text{II.3})$$

where m , the interference constant, modifies the effect of absolute competitor density on foraging success. Clearly, $m > 0$ implies density-dependent feeding success. When an individual's gains deprive others of exactly the same amount of resource, $m = 1$, and equation (II.2) reduces to equation (II.1). If m is not equal to 1, interference changes non-linearly with competitor density. In this case, as Tregenza (1994) has pointed out, the input matching rule need not apply, since feeding gains become a non-linear trade-off between the degree of

interference and patch quality. Therefore, in order to test the hypothesis that competitors obey IFD, gains should ideally be measured directly.

If gains can vary non-linearly with N then so, in principal, can it vary non-linearly with Q , or indeed any other variable. Generalizing (3), therefore, we may write

$$\ln G_i = a + v (\ln Q_i) - m (\ln N_i) + \dots \quad (\text{II.4})$$

Besides N and Q , other x-variables of interest in this paper are the abundance of males sandflies and mosquitoes.

We use two direct measures of gain - the proportion of female flies caught with a bloodmeal, and the proportion of fed females with a large bloodmeal. These are the y-variables of a regression analysis carried out in GLIM (NAG UK Ltd., Oxford), which leads to an estimate of m , v and other coefficients by multiple regression. Since the y-variables are proportions, we used a binomial error structure with a logit link function.

RESULTS

Data on feeding success from the dry season were recorded from 42 of the 62 catches, made on 14 nights of two to four sheds per night. They comprise a total of 29 different sheds, and thus 13 sheds contributed data from both rounds. Wet season data were recorded from 38 of the 40 catches, made on 38 nights, one shed per night.

Sandfly Abundance

The frequency-distribution of males and females of all classes between catches was highly skewed (Fig. II.1) from both the dry season (variance/mean 453 and 376 respectively) and wet season (variance/mean 570 and 657 respectively), and where necessary, catch data were log-transformed to approximate the normal distribution.

During the dry season, sandfly catches varied in size from 30-3117 females and 28-3465 males per shed night. Fowl densities per shed ranged from 2-32. Log(e) male and log(e) female density were highly correlated ($F = 202_{(d.f.1,41)}$, $r^2 = 0.81$). No mosquitoes were caught.

During the wet season, sandfly abundance fell to a range of 3-2299 females and 3-1698 males. During the same period, mosquito densities ranged from 1-661. Fowl densities per shed ranged from 1-26. Log(e) male and Log(e) female density were again highly correlated ($F=114_{(d.f.1,37)}$, $r^2 = 0.75$).

The ranges and geometric means of the proportions of subclasses comprising the female catch per shed night over the wet and dry seasons are shown in Table I.1. Unfed flies predominated in traps.

Figure II.1. Frequency distribution of male (\\) and female (///) *Lu.longipalpis* and female mosquito (≡) abundance from wet and dry season trap catches.

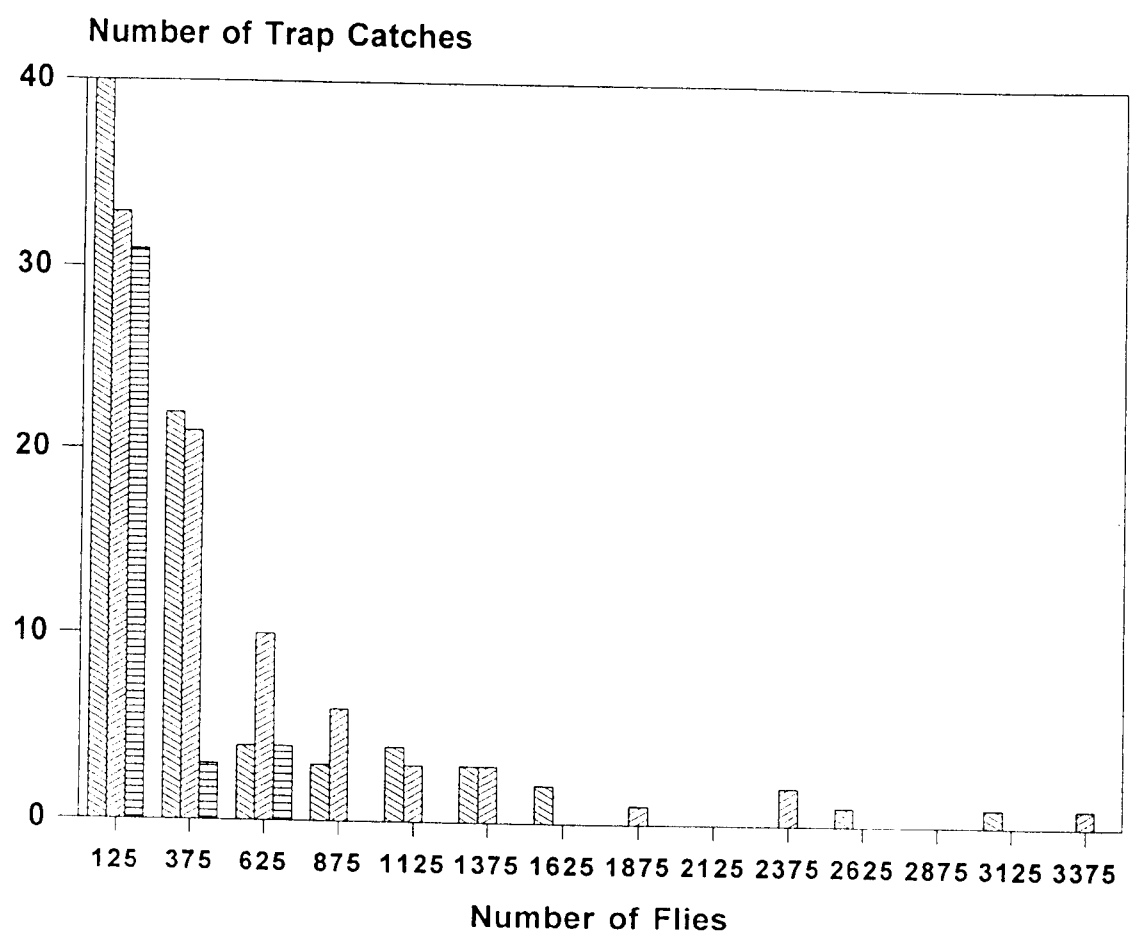


Table II.1. Proportions of females by gonotrophic status, from all traps from dry and wet season.

Dry Season:	Mean (s.e.)	Median	Minimum	Maximum
Unfed	0.759 (0.019)	0.779	0.367	0.957
Small New Meal	0.053 (0.005)	0.049	0.001	0.140
Large New Meal	0.062 (0.008)	0.047	0.013	0.264
Small Old Meal	0.045 (0.005)	0.036	0.006	0.193
Large Old Meal	0.031 (0.004)	0.026	0.000	0.093
Gravid	0.050 (0.011)	0.039	0.000	0.467

Determinants of Feeding Success

Sandfly behaviour may be influenced by the changing climate between dry and wet seasons. In particular, it was considered likely that high rainfall on often poorly-constructed sheds, and resulting leakage, would lead to increased variation in trapping efficiency and unknown trapping bias. For this reason, the analyses of dry and wet season catches were conducted separately.

Dry Season. To investigate the determinants of G_i , the following model maximal for the

data collected was initially fitted by multiple ANCOVA:

$$\ln (G_i) = a + b(\ln M_i) + c(\ln Q_i) + d(\ln N_i) + e.D_j \quad (\text{II.5})$$

where M_i is the number of males caught in shed i , and D_j is a 14-level factor for the day on which the catch was made. Regression terms were removed by a process of backward elimination, beginning with the parameter with the poorest t -value and continuing until the removal of any further terms resulted in a significant increase in error by Chi-squared analysis (Crawley 1993).

Anticipating the model fitted by GLIM with the logit link function, the most obvious index of gain, G_i , is the odds of a female getting a new bloodmeal (odds ratio, OR_N). However, unlike the data on other bloodsucking insects, none of the parameters in our model, including female density (Fig. II.2a), had a significant influence on OR_N . Dye *et al* (1991) show that the position of the trap with respect to the roosting fowl affects the proportion of flies found bloodfed. Variation in trap position in the present study might therefore have obscured any relationship between host abundance and OR_N . To get around this, new meals were divided instead into small and large, and the odds of a female getting a large meal (OR_L), fitted as the index of G . The resultant minimum adequate model (Table II.2, Fig. II.2b) is:

$$\ln (G_i) = a + v(\ln Q_i) + m(\ln N_i) \quad (\text{II.6})$$

The parameter value m from this model can be conveniently compared to m from Sutherland's linearized interference model (Eq. II.3). The estimate is negative and significantly different from zero, demonstrating that *per capita* feeding success is indeed reduced by

increasing female density.

Conversely, the host parameter estimate, ν , is positive, indicating that increasing host abundance, Q , increases feeding success. The estimate of ν is also significantly different from 1 (95% C.I. 0.226 - 0.549), indicating that the influence of host abundance on feeding success is less than linear. This is not allowed by Sutherland's model (Eq. II.3), which would therefore produce a biased estimate of m when Q and N are correlated. Finally, the absolute values of $|\nu|$ and $|m|$ are significantly different ($t=6.87_{(d.f. 82)}$; $p<0.001$), implying that maintaining a constant ratio of N/Q over changing abundance of N and Q is not sufficient to maintain constant feeding success.

Table II.2. Parameter estimates from the minimum adequate model for the dry season data (Eq. II.6), and with only the night factor (D) and female abundance (N) (Eq. II.7).

	Parameter	Standard	Adjusted	
PARAMETER	Estimate	Error	Chi-Squared	p
Intercept	3.355	0.3372	/	/
Ln Host (ν)	0.388	0.08259	10.39	<0.005
Ln Females (m)	-0.6949	0.05305	30.09	<0.001
Intercept	3.302	0.5453	/	/
Night (D)	/	/	2.19	>0.1
Ln Females (m)	-0.5981	0.0857	18.79	<0.001

Figure II.2a. Proportion of the total female *Lu.longipalpis* with a new bloodmeal in dry season trap catches versus ln total female abundance in traps.

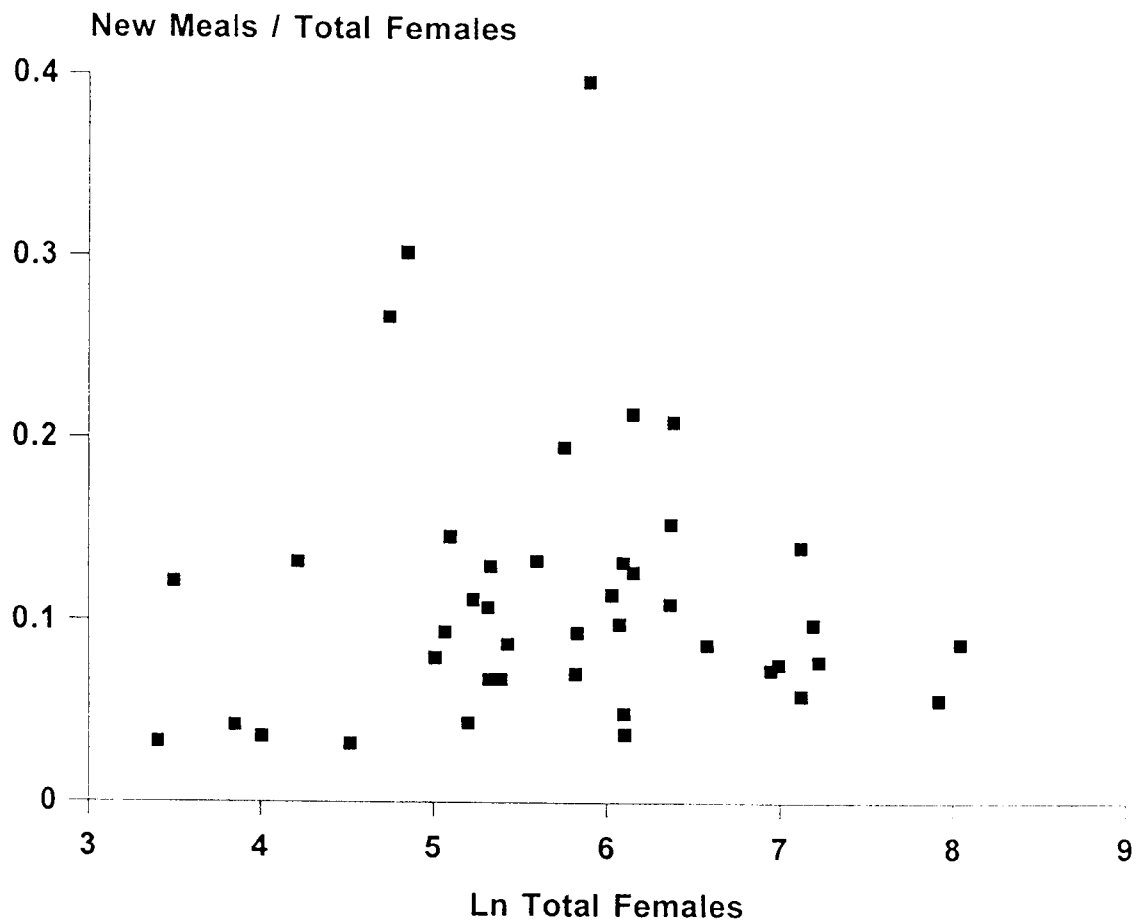
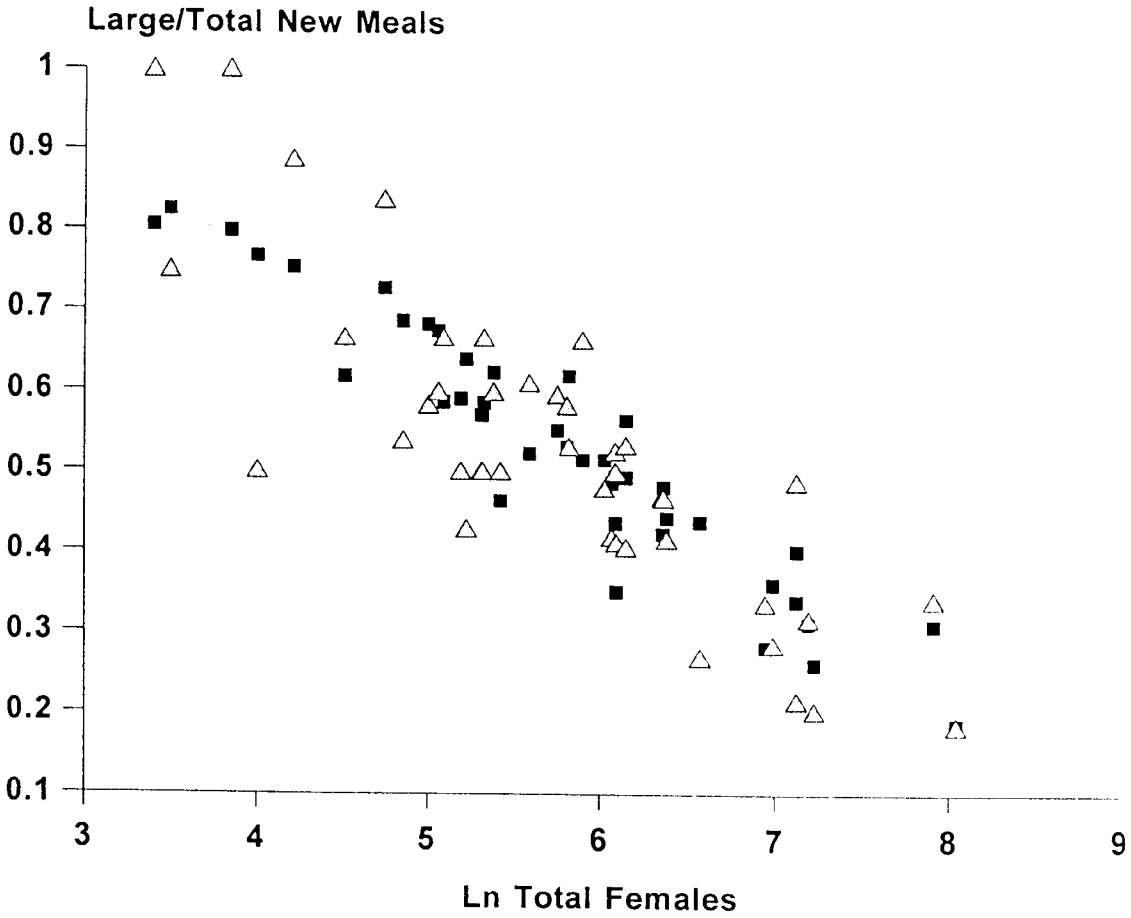


Figure II.2b. Proportion of female *Lu.longipalpis* in dry season trap catches with new bloodmeals that are large versus ln total female abundance in traps; observed (Δ) and fitted values (\square) from the minimum adequate model (Eq. II.6).



To test that m has a detectable effect on feeding success between sheds on individual nights, and not only as population density is fluctuating between nights, a second regression model was fitted with just N and the night factor, D_j , in order to control for fluctuations in mean female and fowl abundance between nights (Table II.2):

$$\ln (G_i) = a + e.D_j - m(\ln N_i) \quad (\text{II.7})$$

The resulting estimate for m remains significant and close to its original value from equation (II.6).

Wet Season. Proceeding as for the dry season analysis, the following maximal model was first fitted:

$$\ln (G_i) = a + b(\ln M_i) + c(\ln Q_i) + d(\ln N_i) + e(\ln C_i) + f.I_j \quad (\text{II.8})$$

where C_i is the number of female mosquitoes competing with sandflies at each shed catch. There is no factor for trapping night, N_j , as each shed was trapped on a separate night. Unlike the dry season data, however, all but two of the ten sheds were trapped four times, and the regression includes the ten-level shed factor I_j , for the shed in which each catch was made.

The regression was reduced to the following minimum adequate model (Table II.3):

$$\ln (G_i) = a + l(\ln M_i) - m(\ln N_i) - e(\ln C_i) + f.I_j \quad (\text{II.9})$$

where the shed factor estimates reduced from ten to two levels, each comprising five sheds,

Table II.3. Parameter estimates from the minimum adequate model for the wet season data (Eq. II.9).

PARAMETER	Parameter Estimate	Standard Error	Adjusted Chi-Squared	p
Intercept	1.924	0.4183	/	/
Shed(2) (<i>f</i>)	-0.7956	0.1214	12.78	<0.005
Ln Males (<i>l</i>)	0.4161	0.1071	5.89	<0.025
Ln Females (<i>m</i>)	-0.5721	0.1188	8.6	<0.005
Ln Mosquitoes (<i>e</i>)	-0.1096	0.0337	5.25	<0.025

which had mean G significantly different from each other (Chi-squared: $12.78_{(d.f.1)}$, $p < 0.001$).

As for the dry season (Eq. II.6), increasing densities of sandflies result in a decrease in the proportion of large-fed sandflies, and the estimate m is not significantly different between the two periods ($t=0.82$, $p > 0.01$). In addition, a weak but significant reduction in feeding success was also detected for increasing mosquito density. This mosquito effect, e , is significantly smaller than m ($t=5.34$, $p < 0.001$).

The major difference between the dry season and wet season results is that the positive influence of host density, Q , on feeding success was replaced by a male density effect, M . However, it seems possible that M and Q are substituting for one another. The two parameter estimates (v and l) are not significantly different from each other between the two

trapping periods ($t=0.4$, $p>0.05$). The relationship between l and m in the wet season is also similar to v and m in the dry season, having an opposite sign, and of smaller magnitude (though, unlike the dry season, not significantly so: $t=1.1$, $p>0.05$).

As expected, there was much more inherent variability in the wet season data. Both the amount of error explained, and the standard errors for the parameter estimates for the wet season analysis are larger than for the dry season, possibly as a result of changes in meteorology.

Bloodmeal and Fly Size

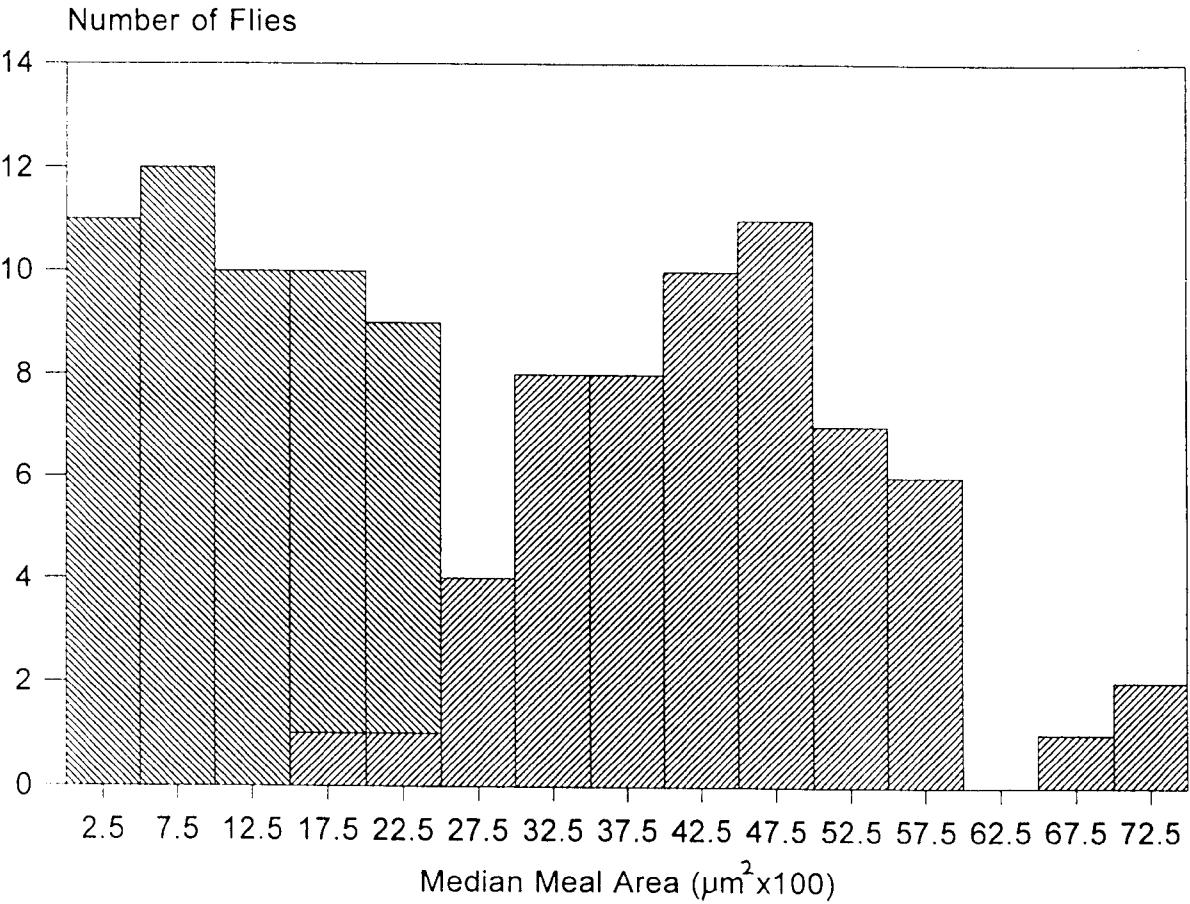
The frequency distribution of new meal sizes taken from a random sample of shed catches clearly shows a bimodal distribution, dividing between what were identified in the field as small and large meals (Fig. II.3). There is a decline in the frequency of small meals from zero to $0.25\mu\text{m}^2$ in area, and between the range of $0.15\mu\text{m}^2$ to $0.75\mu\text{m}^2$, large meals are approximately normally distributed.

There is no significant difference in wing length between the small and large meal classes (one-tailed t-test: $t=1.4_{(d.f.93)}$, $p=0.17$). There is also no correlation between meal size and wing length within the small meal class ($F=2.23_{(d.f.1,43)}$, $p=0.142$, $r^2=0.027$). However, there is a positive correlation between bloodmeal size and wing length among flies which took large meals ($F=6.16_{(d.f.1,51)}$, $p=0.016$, $r^2=0.09$), most probably due to the ability of larger flies to take bloodmeals of larger maximum size.

Forty six percent (43/93) of small new meals stored in alcohol for morphometric analysis did not have a detectable compact bloodmeal suitable for measurement on later examination. It is assumed that these bloodmeals were too small at the time of feeding to clot and allow the formation of a coherent peritrophic membrane. However, in order to avoid bias,

if this assumption is incorrect, these flies were not used in the frequency distribution.

Figure II.3. Frequency distribution of bloodmeal sizes (measured in plan) of all bloodfed female *Lu.longipalpis* from a random sample of trap catches from the dry and wet season; flies classified as small meals (\\) and large meals (///) on the day of capture.



DISCUSSION

Determinants of Density-Dependent Feeding Success

The maximal regression model for the dry season data, using OR_L as the index of G , conveniently reduces to estimates of the coefficients of Q and N : in the final, minimal model a decreasing proportion of newly-fed flies get a large meal as N_i increases, whilst increasing Q_i goes some way towards counteracting the effect (Table II.2). The effect of female density on feeding success is not significantly different between nights, nor is there any evidence from the dry season data that increasing numbers of males alter a female's chance of taking a large meal. In the wet season, however, there is no detectable effect of Q_i on G . Rather, M seems to substitute for it. Certainly, the coefficients of Q (Table II.2) and M (Table II.3) are similar in magnitude and in their relationship to m . It may be that the mechanisms by which Q and M affect G are similar, and confound each other in the analysis, causing one or other parameter to fall out of the minimal model.

The findings differed from published results of cage experiments carried out with other bloodsucking insects (e.g. Waage & Nondo, 1982), as no evidence was found for a reduction in the odds of a female obtaining any class of new bloodmeal (large or small). In order for a female to fail to take even the smallest bloodmeal, it must be interrupted during its pre-feeding behaviour on the host, and it may be that natural biting densities of female *Lu. longipalpis* are insufficient to interrupt detectable numbers of females at this stage.

Despite the greater variability in the wet season data, the estimate of the effect of female sandfly density on *per capita* feeding success is similar to that for the dry season. The mosquito effect is relatively small, and, together with relatively low mosquito abundance in chicken sheds,

argues that they are unlikely to have a great impact on sandfly numbers in our study area.

Mechanism of Density-Dependence

There are two plausible mechanisms by which the density of female *Lu.longipalpis* could influence feeding success:

(i) *Direct female-female competition.* Females, concentrated at feeding sites on the host, might interrupt one another during the course of a feed. In this case, an individual female would be expected to stand a constant chance of being interrupted by another, as other females arrive at random during feeding, and the expected frequency distribution of meal sizes, while being shifted left of the 'non-competitive' distribution, would therefore have a single peak. Interrupted flies might also be expected to be smaller than uninterrupted, being more frequently displaced by their larger rivals.

The data support neither of these predictions (Fig. II.3). There is therefore no discernible female-female competition occurring, at least on the basis of size. This agrees with Waage and Davies (1986), who found that less than 1% of interrupted feeds resulted from direct interaction between the horseflies in their study. They argue that free-living parasites will rarely achieve such densities, as hosts are too large and numerous relative to parasite population sizes.

(ii) *Host-mediated female-female competition.* Is the more commonly accepted mechanism of density-dependent feeding success in blood-sucking diptera. Pain is greatest at the beginning of a bite, and diminishes as the rasping action of the mouthparts ceases. Thus the likelihood of a host taking defensive action decreases with time after initiation of a feed. This predicts the

declining frequency of small bloodmeals seen from $0\mu\text{m}^2$, while the large bloodmeals are normally distributed, suggesting that these flies are leaving at will once sated (Fig. II.3). This bimodality is similar to that seen by Waage and Davies (1986) for the horsefly *Hybomitra expolicata*, for which they demonstrate a host-mediated mechanism.

A detectable difference in the size of flies between the small and large meals would not be expected, as any such difference would have to be driven by size differences either in painfulness of the bites or ability to continue feeding during host defensive behaviour, either of which is likely to be trivial. Furthermore, the significant correlation between wing length and meal size in the large but not the small meal class argues that interruption of feeding occurs predominantly in the small meal class.

Costs of Density-Dependence

Overall, host-mediated interference seems to be the major mechanism of female-female competition. There are two potential costs of such interference to female fitness:

(i) *Reduced fecundity per gonotrophic cycle.* Carneiro *et al* (1993) allowed one cohort of female *Lu.longipalpis* to feed to repletion, while a second was interrupted before the abdomen had become distended - the same criterion used in our study to classify large and small feeds. After seven days (ie before oviposition) both cohorts were offered a second meal, but while 100% of partially fed flies re-fed, only 25% of flies originally fed to repletion fed again. Abdominal distention, and thus willingness to re-feed, also correlated with egg production: 100% of fully-fed flies produced eggs, with a mean egg batch size of 75. Only 6% of underfed flies produced eggs, and of those, the mean egg batch size was only 20. Elnaiem *et al* (1992) also showed that

willingness of *Lu.longipalpis* to re-feed within a gonotrophic cycle increases with the passage of time post-feed, as the bloodmeal is excreted and the midgut shrinks. Also, Ready (1979) found that the number of eggs produced by a female *Lu.longipalpis* is directly proportional to the amount of blood ingested.

As already discussed, a female *Lu.longipalpis* which begins to feed and provokes a host response causes the interruption of other flies feeding on the same host. Some of these will already have fed sufficiently to distend the abdomen, and may have lost the motivation to refeed, before maximising their bloodmeal size. Therefore, the average meal size per gonotrophic cycle would be smaller as female density increased, resulting in reduced fecundity per gonotrophic cycle.

(ii) *Increased mortality rate per gonotrophic cycle.* Those flies which *are* motivated to re-feed will encounter additional risks associated with feeding. Even the act of immediate re-feeding, without postponement to the following night, must have an associated risk. Previous workers have shown mosquito mortality rates during feeding in cage experiments with unrestrained animals of 8.7% with rabbits (Waage & Nondo 1982) and between 9 and 27% with a range of ciconiiform birds (Edman *et al* 1972, Table 1, experiment 2).

In addition to a cost to the task of refeeding, a number of flies apparently fail to take anything more than a small meal in a single night. Evidence for this comes from trap catches of flies with small, old bloodmeals. These are interpreted as flies that had taken a small meal on the previous night and then failed to refeed. During the dry season, for example, an average of 4.5% of flies caught in traps were of this category, with a range of 0.6% to 19.3% (Table II.1). This is probably an under-estimate, as many small meals are small enough to become rapidly undetectable. This is suggested by the large percentage of flies with small meals which were

undetectable after storage in alcohol.

Female *Lu. longipalpis* which do not obtain a large meal on the first night must bear the cost of resting during the day and, if they still have an appetite, seeking a host on the following night in order to refeed. Since flies do not rest in chicken sheds by day (Quinnell & Dye 1994a) the risks could be considerable. However, it is unclear exactly what proportion of flies caught with small new meals would, if they had not been trapped, have succeeded in refeeding on the same night. Thus a potentially important cost to female fitness remains unquantified.

The Ideal Free Distribution

Thinking about the behaviour behind the ecology, we can consider this cost in terms of the Ideal Free Distribution. IFD theory predicts that costs to fitness should be minimized through the pattern in which females distribute themselves over all available hosts. Whether this actually occurs can be tested on two spatial scales. The first test uses a direct measure of gain, as is preferred (Tregenza 1994), though the second does not.

(i) *Between sheds within night.* Clearly feeding success varies between sheds (Tables II.2 & II.3, Eq.s II.6 & II.9, Fig. II.2b). Does this, however, simply reflect changing population densities between nights, over which individual flies can have no control, or are flies on any one night incurring costs by distributing themselves other than by IFD?

This can be investigated using the dry season data, where more than one shed was trapped on each night. The inclusion of the night factor, D , in equation (II.7), accounts for mean day-to-day differences in female feeding success. If females are distributed so as to maximize feeding success across all patches on any one night, then the estimate of m (Eq. II.7, Table II.2)

should not be significantly different from zero. In fact, the magnitude of m is reduced only slightly, still significantly different from zero, and still in the same direction. In other words, we find a significant reduction in feeding success between sheds on any one night associated with increasing female density, closely approximating the overall trend and suggesting that flies are not distributed to maximize individual gains.

Intuitively, the sub-optimal distribution of females between sheds seems likely to be a result of the way in which males distribute themselves, females following the male distribution in response to pheromone cues. Certainly, between sheds, male and female distributions are highly correlated.

However, the predicted distribution of flies from basic IFD theory (Eq.s II.1, II.2 & II.3) rests on two key assumptions. Firstly, flies are assumed to be equal competitors. If this is not so, different phenotypes may be segregated in patches of different values in different numbers (Parker & Sutherland 1986). The fact that there is no significant difference in size between flies with small and large meals suggests that, at least with size as an index, flies are indeed no different in their competitive abilities.

Secondly, there should be no cost for travel between resource patches. IFD theory predicts that flies should only leave a resource patch if the increased gains at the new patch outweigh the costs of travel. Our study was restricted on the spatial scale, with no shed on any one night being more than 50m from its nearest neighbouring study shed, and *Lu.longipalpis* is known to be capable of travelling hundreds of meters in a night (Dye *et al* 1991, Morrison *et al* 1993). It is still possible that female distribution is sensitive to distance to the nearest alternative host, but this is hard to investigate, and has been the source of some recent debate (Kennedy & Gray 1993, Ånström 1994).

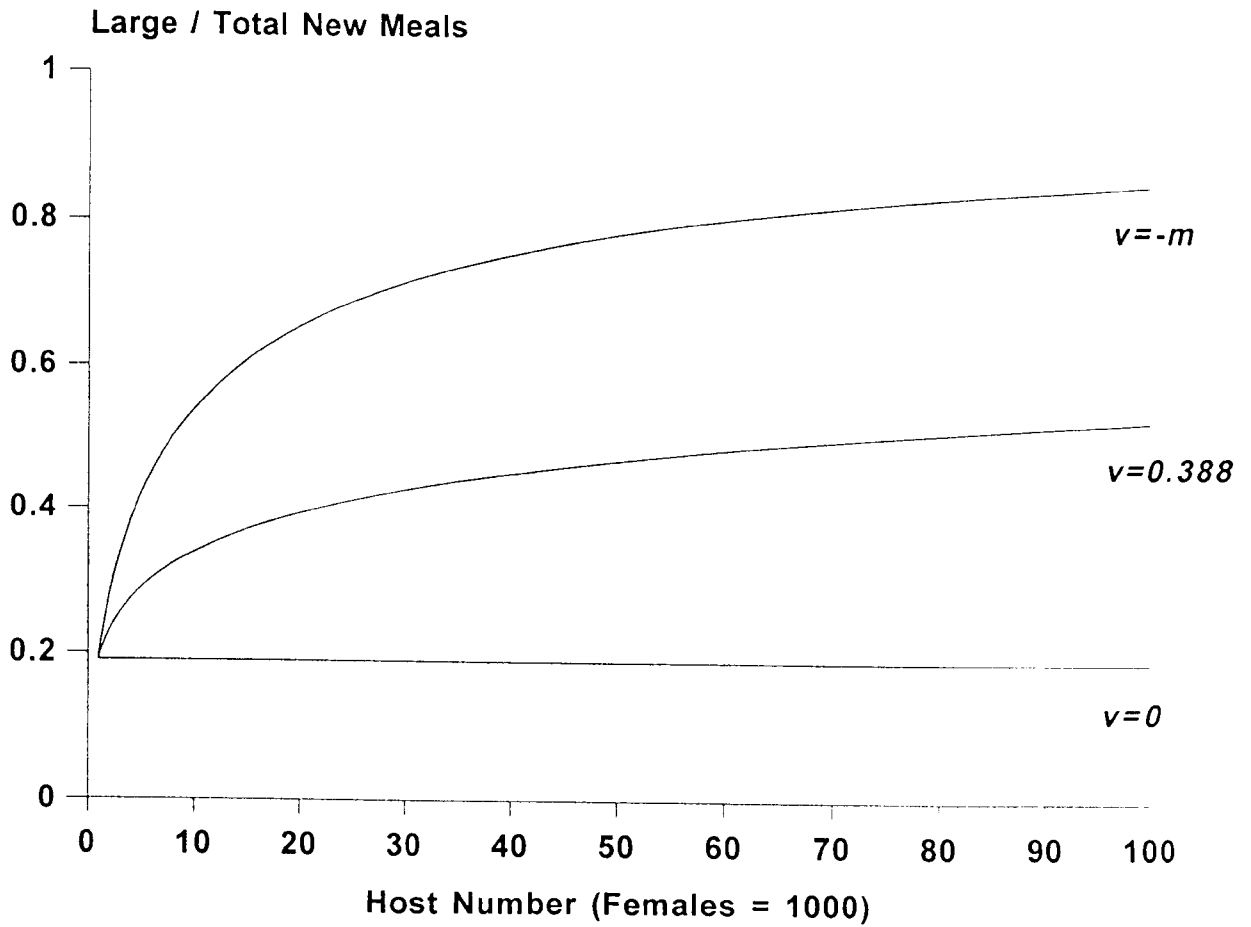
(ii) *Within sheds*. One way to get around the interpretive difficulties of travel costs is to look at the behaviour of females within sheds, where travel costs should be negligible. Inside the shed, individual fowl in a roost form the patches, Q , between which a female has to choose, and patch quality may be defined as ease of feeding, the major determinant of which we identify as host tolerance to bites.

If we assume that our index of body size is sufficient to measure the absolute quality of hosts in terms of ease of feeding, then v must equal $-m$ in order to maximise feeding gains, i.e. the null hypothesis is that gain depends on Q/N . In fact, our estimate of v is significantly smaller than m in absolute terms, indicating that females are distributing themselves non-randomly between hosts: they are not feeding evenly across all the available fowl in a shed, but concentrating on only a fraction (Fig. II.4).

Casual observation suggests that males are overdispersed between hosts within sheds, with one or two fowl supporting large aggregations and other males sparsely distributed over the remaining fowl and shed structures. The utilization of additional hosts within the shed (at the rate v , equation II.6) could thus be thought of as resulting from the formation of satellite leks as male density increases, rather than as a search for better feeding sites. In this context, we note that M replaces Q in the wet season data. However, it is also likely that our index of Q is not sufficient to predict the true value of hosts in terms of ease of feeding, and it is this discrepancy which causes v to be smaller than m in absolute terms.

Whether fowl are unequally attractive to females in terms of ease of feed (ie Q) or simply unequally attractive as a result of lek distribution cannot be discovered from the data; it requires the measurement of feeding gains from individual fowl (Tregenza 1994). In reality, the two are probably inextricably linked. Since males lek on the backs of chickens, host defensive behaviour

Figure II.4. The change in feeding success, G , within shed, with a constant female density, N , and increasing host density, Q , calculated from the minimal model (Eq. II.6) with three values of v : (a) $v = 0$; (b) $v = -m$; (c) $v = 0.388$.



also disrupts lekking aggregations (pers. obs.) which may work as a non-sophisticated method of selecting the most passive fowl (ie the highest quality patches): the least restless allowing the build-up of the largest leks.

Extending these results to feeding patterns on canids and humans, whatever the mechanism, the aggregation of feeding activities on a subset of hosts has important consequences for disease epidemiology, particularly if, as suggested, it is the most listless hosts which are most fed upon, since these are likely to be diseased individuals.

Sylvatic Versus Domestic Environments

Much of the behaviour of *Lu.longipalpis* will have been shaped by its more ancient relationship with the sylvatic environment. Here, where hosts and sandflies are widely dispersed and unpredictably distributed (Lainson *et al* 1990), it is easy to see how pheromone-mediated location to the feeding site would be economical to the female, lowering the travel costs of finding a host and mate. Similarly, it is likely that sylvatic males would find it more economical to locate pheromone-producing males at a host site, rather than searching for other, unoccupied, but less easily located hosts. Thus, although males should be more concerned with locating a host at which to signal, rather than joining other males with which they must compete for mates (Harvey & Bradbury 1991), they may never have needed to evolve a habit of distributing themselves evenly over hosts, say by being repelled from leks at high pheromone concentrations.

However, recruitment of females (and males) to pheromone appears to hold disadvantages in the peridomestic arena, at least between sheds, where a superabundance of flies in high density aggregations leads to a reduction in female feeding success (and therefore in the average fitness of matings that a male secures). The evident excess of hosts implies that flies

could reduce density-dependent costs by distributing themselves more evenly with what seem likely to be relatively small travel costs. The response of male and female *Lu.longipalpis* to pheromone could therefore be regarded as a maladaptation in the peridomestic environment.

CHAPTER III

PHEROMONES, KAIROMONES AND AGGREGATION

BEHAVIOUR IN A FIELD POPULATION

OF THE SANDFLY *Lutzomyia longipalpis*

SUMMARY

- (1) Host kairomones and a male pheromone are thought to be important in the formation of mating/feeding aggregations of the sandfly *Lutzomyia longipalpis*.
- (2) Stimulated by interest in the development of a semiochemical-baited trap for fly control, a technique was developed to mark flies with minimum disruption of their natural behaviour, and employed in a set of field experiments to investigate the role of host and fly factors in aggregation dynamics.
- (3) Males arrived at aggregations earlier than females, at a rate dependent on the abundance of resident flies and hosts. The immigration rate of females was dependent on fly abundance alone.
- (4) The emigration rate of males decreased as fly and host abundance increased. The emigration rate of females was greater than males, and increased with host abundance, but decreased with female abundance.
- (5) We argue that male behaviour maximises mating success, whereas female behaviour depends on the rate of bloodfeeding and the desire to minimise travel costs.
- (6) Between nights, most males returned to the site of their previous night's activity, suggesting that flies may memorize a "familiar area map".
- (7) These results raise the possibility that, without the addition of pheromone baits, insecticide spraying programmes which do not achieve blanket coverage of aggregation sites would not significantly reduce the fly population, and might increase parasite transmission between susceptible hosts.

INTRODUCTION

As discussed in Chapter I, the major foci of peridomestic fly activity are animal pens, and in particular chicken sheds, where catches are on average ten-fold greater than in houses (Quinnell & Dye 1994b). Quinnell & Dye (1994a,b) explain this difference as the result of host accessibility: typically, chicken sheds comprise a palisade of wooden poles set several centimetres apart; houses are of solid adobe walls with close-fitting doors and shutters. However, the distribution of males and females between similarly-constructed animal pens is also typically highly overdispersed (Quinnell & Dye 1994b; Chapter II), with the majority of the flies caught at only a few host sites. This cannot be explained by differences in accessibility.

Of the factors which might affect the distribution and development of aggregations between animal pens, semiochemicals have received considerable attention, with a view to producing a trap which would attract flies to a bait in numbers similar to those seen in chicken sheds (Brazil *et al* 1989; Ward *et al* 1990). Two classes of semiochemicals have been studied: host kairomones and a pheromone produced by the male *Lu.longipalpis* (Lane & Ward 1984, Lane *et al* 1985, Phillips *et al* 1986). The former have been well-studied with other bloodsucking diptera (e.g. Bennet *et al* 1972; Hall *et al* 1984; Vale *et al* 1988; Gillies 1980), and cage experiments in the laboratory using virgin, unfed *Lu.longipalpis* females have demonstrated that live hamster volatiles are attractive (Oshagi *et al* 1994). Similar experiments with hexane extracts of male pheromone have demonstrated attraction and arrest of unfed virgin females (Morton & Ward 1989a; Morton & Ward 1989b).

The extrapolation of these laboratory results to the field situation may be limited for a number of reasons. First, experiments have only been conducted over tens of centimetres, whereas attraction may occur over much greater distances in the field (Alexander 1987; Dye *et*

al 1991; Morrison *et al* 1993). Second, the use of extracted material precludes physical interactions between males, and between males and females, which communicate over short distances by wing beating (Ward *et al* 1988), all of which might affect male pheromone production. Finally, there is a danger that inbred laboratory colonies will differ from the wild-type in their behaviour (e.g. Poppy 1990, on moths).

To begin addressing these problems, Dye *et al* (1991) carried out a series of experimental and observational studies of *Lu.longipalpis* aggregations in animal pens in the field. Their results led them to infer that pheromone traps could not succeed by significantly increasing recruitment of females to a bait. These inferences depend on the assumption that kairomones and pheromones are influencing the immigration rate only. However, the dynamics of mating/feeding aggregations depend on both immigration to, and emigration from the host site. Intuitively, male and female sandflies, which have different objectives at the aggregation, may have different responses to the factors mediating their rates of emigration in addition to any effect on immigration. A second assumption, in the case of the longitudinal study of shed colonization, is that flies are habitually returning to the same shed from night to night. If this is so, different biological priorities between the sexes from one night to the next might again result in differences in behaviour.

In this study, we therefore extend the earlier field work on the dynamics of aggregations in natural populations of *Lu.longipalpis*. We develop a new technique for fly self-marking which aims to affect the natural behaviour of flies in the field as little as possible, and then use it to study the determinants of male and female immigration and emigration rates.

MATERIALS & METHODS

Study Area and Timing

The study took place in Salvaterra district on the island of Marajó, described in Chapter II. Sandfly aggregations in chicken sheds from two villages were studied. Pingo d'Agua (PA) and Vila Ceará (VC) were approximately 2km apart, separated by fields and dense secondary forest, and both were broadly linear in form. Homesteads were typically planted with fruiting trees. Domestic animals other than fowl - mainly dogs and pigs - were common.

Trapping was conducted from 22 July - 11 August 1992 (experiment 1), 18 May - 5 June 1993 (experiment 2) and 27 June - 13 July 1993 (experiment 3). All dates fall within the dry season on Marajó Island.

Identification of Sandflies

Female *Lu.longipalpis* were routinely identified on external morphology, although occasional checks were made by examining spermathecae (Ryan 1986). Males of the population of *Lu.longipalpis* on Marajó Island bear a single pale spot on tergite IV. Only diterpenoid-like pheromones have been isolated from males in this area (Ward *et al* 1988), suggesting the presence of only a single sibling species.

Self-Marking Technique

Schlein (1987) sprayed dyed sugar solutions onto foliage surrounding burrows of

Psammomys obesus (Cretzschmar), an important maintenance host of the sandfly *Phlebotomus papatasi* (Scolopi). As a result, approximately 30% of flies caught in nearby traps had ingested sufficient sugar for the colour to be clearly visible in the crop. We adapted this technique to make the sugar-mark source portable, and therefore easily introduced or removed from the experimental arena. White cotton sheets in 1m² squares were impregnated with a saturated sugar solution mixed with one of a range of colours of a locally available liquid food colouring ("Carmil", Carmil Produtos Alimentícios Ltda., Rio de Janeiro). Coated sheets (marking sheets) were wrung out and left to dry before use in marking experiments.

Experiment 1: Aggregation at Male and Female Baits

Two newly-constructed chicken sheds (1m³) were set 5m apart in PA, across the direction of the prevailing wind and approximately 15m from the nearest resident shed. At 1800 hours, a single chicken in a wire cage, sheathed in sandfly-proof netting, was placed in each shed. In addition, one of the cages contained 120 live male sandflies caught the night before. A CDC miniature light-suction trap (CDC trap) was run concurrently in each shed from 1800-0630 hours. Flies caught in the CDC traps were counted as male or female. Seven repeats were made, with three or four days between each, alternating the site of the male bait. The experiment was then repeated on five nights using a bait of 120 female *Lu.longipalpis*.

Experiment 2: Aggregation Dynamics Within Nights

Between 1800 and 2100 hours, either a blue or red marking sheet was hung in each of a pair of neighbouring sheds in PA. At 2100 hours, sheets were removed, and a CDC trap was

installed in each shed. Collecting bags on the CDC traps were changed at hourly intervals, up to midnight (traps 1-3), when a bag was left in place until 0630 hours (trap 4). Flies in each bag were examined at x8 magnification immediately upon collection, and counted as male or female, marked or unmarked, red or blue. The experiment was repeated four times at three pairs of sheds, one pair per night in rotation. Pairs of sheds on any one night were 30-50m apart. Catches were excluded from the analysis where a CDC bulb or battery had failed during the course of the night.

Migration to and from one shed. From 1800-2100 hours, with the marking sheet in place, resident unmarked flies (U) can become colour-marked (C). Both U and C can emigrate from the shed, but all immigrants are U. Changes in the abundance of U and C over time can therefore be described as follows:

$$\frac{dU}{dt} = (\iota - \epsilon - \mu)U \quad (\text{III.1})$$

and,

$$\frac{dC}{dt} = \mu U - \epsilon C \quad (\text{III.2})$$

where ι , ϵ and μ are, respectively, per capita rates of immigration, emigration and marking. The changes in U and C during this period are governed by several factors, and the influence of each factor is difficult to quantify. However, the solutions to equations (III.1) and (III.2) after 2100

hours, when the marking sheet has been removed and CDC-traps installed, are:

$$U_t = U_{21} \cdot e^{(1-\epsilon)t} \quad (\text{III.3})$$

and,

$$C_t = C_{21} \cdot e^{-\epsilon t} \quad (\text{III.4})$$

where U_{21} and C_{21} denote, respectively, the abundance of unmarked and colour-marked flies after 2100 hours.

CDC-trapping has a negligible effect on the abundance of flies in sheds (R.J. Quinnell and C. Dye, unpublished observations), so the abundance of C over the trapping period depends only on the emigration rate. Linearizing equation (III.4) by taking logarithms:

$$\ln C_t = \ln C_{21} - \epsilon t \quad (\text{III.5})$$

Moreover, assuming that C and U have the same emigration rates, their relative abundance depends on the immigration rate alone:

$$\frac{U_t}{C_t} = \frac{U_{21}}{C_{21}} \cdot e^{\nu t} \quad (\text{III.6})$$

which can again be linearized by log transformation:

$$\ln \left(\frac{U_t}{C_t} \right) = \ln \left(\frac{U_{21}}{C_{21}} \right) + \nu t \quad (\text{III.7})$$

So, by using the data from trap catches 1-4 together with equations (III.5) and (III.7), we have a simple method of estimating ϵ and ι . We can then investigate the influences of host, male and female abundance on these rates, by exploring variation in host and fly abundance between sheds.

The total number caught in CDC traps from 2100-0630 hours was used as the index of *Lu.longipalpis* abundance in each shed. The number and size of fowl in each shed were recorded at the beginning of each trapping night. Fowl were weighted a priori for size by scoring adults as 1, pullets as 0.5 and chicks as zero, to arrive at an index of host abundance.

Migration between two sheds. Alternative estimates of ϵ and ι can be derived from the movement of marked flies between sheds during the night. Since each experiment-night comprises two sheds, each with a different coloured sheet, it is possible to identify flies which have visited one shed ('home'), become marked and then exited to the other shed ('away'). These flies have carried out two actions: they have left the home shed and entered the away shed. The total numbers of immigrant marked flies caught at away sheds can therefore be used as an index of emigration rate from the home shed and immigration rate to the away shed. Regression analysis can then be used to model the effects of host and fly abundance on immigration and emigration rates.

Experiment 3: Aggregation Dynamics Between Nights

In order to investigate the movement of flies between sheds from one night to the next, pairs of sheds were again classified as 'home' or 'away'. On night 1, a marking sheet was hung from 1800-0630 hours in the home shed, and a CDC trap was run concurrently in both home and

away sheds. On night 2, marking sheets were removed, and CDC traps were run alone in home and away sheds from 1800-0630 hours. The experiment was run - two nights on, two nights off - in each of four pairs of sheds, two pairs each in PA and VC, separated by distances of 4, 30, 100 and 130 meters. Four repeats were conducted for each pair, and on each repeat the home and away shed status within a pair was reversed. Repeats were excluded from the analysis if CDC traps failed on nights 1 or 2.

RESULTS

Experiment 1: Aggregation at Male and Female Baits

The female bait consistently and significantly increased female abundance (geometric means: treatment = 29.3, control = 9.1; two-tailed t-test: $t_{1,8}=2.68$, $p=0.044$; Table III.1), and, not significantly, male abundance (geometric means: 27.9 vs. 12.9; $t_{1,8}=1.96$, $p=0.091$). The male bait produced a more dramatic increase (geometric mean females: 91.9 vs. 6.4; $t_{1,12}=3.88$, $p=0.006$; geometric mean males: 64.3 vs. 3.6; $t_{1,12}=5.03$, $p=0.002$; Table III.1), significantly greater than the effect of the female bait (males: $\chi^2_1=26.0$, $p<0.001$; females: $\chi^2_1=17.9$, $p<0.001$), confirming the findings of Dye *et al* (1991).

Experiments 2 & 3: Sandfly and Host Abundance

The geometric mean abundances of males, females and fowl per shed, were 113.1, 103.1 and 5.9 respectively during experiment 2, and 69.4, 42.8 and 6.3 during experiment 3. Catches of males (two-tailed t-test: $t_{1,18}=2.01$; $p=0.044$) and females ($t_{1,18}=3.03$; $p=0.004$) in experiment 2 were significantly larger than in experiment 3, despite the fact that we only trapped from 2100-0630 hours. However, there was no difference in host abundance between the two experiments ($t_{1,18}=0.42$; $p=0.68$).

Fly abundance (males+females) in experiment 2 increased non-linearly with host abundance (Fig. III.1). On fitting a quadratic term for host abundance, r^2 increased significantly from 57.7% to 77.3% ($F=14.7_{(1,19)}$, $p<0.01$). The same result was obtained when males and females were analysed separately (males: $F=7.6_{(1,19)}$, $0.05>p>0.01$; females: $F=9.6_{(1,19)}$, $p<0.01$).

Figure III.1. Total catch (males + females) from 2100-0630 hours versus host abundance for each shed-night in experiment one. The line represents the fitted values from regression with the quadratic term host^2 .

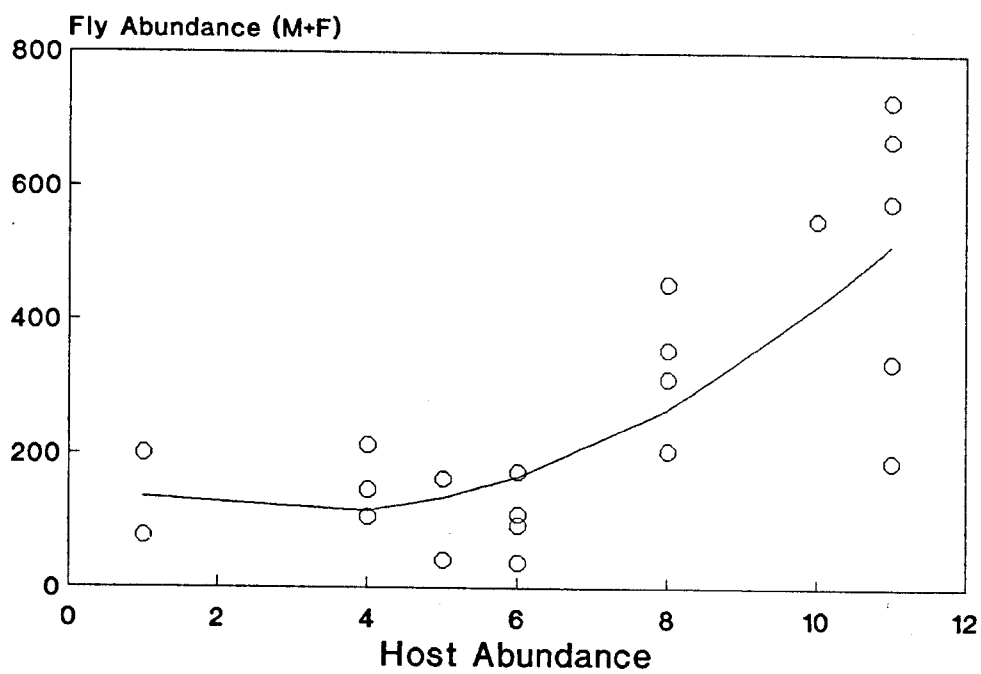


Table III.1. Number of males and females caught at experimental chicken sheds with and without baits of 120 male or female *Lu.longipalpis*.

Trap Night	+ Bait			- Bait		
	♂	♀	♂+♀	♂	♀	♂+♀
120 Females Attracting						
1	26	26	52	15	17	32
2	35	26	61	11	10	21
3	36	50	86	33	25	58
4	52	40	92	11	5	16
5	10	16	26	6	3	9
Σ	159	158	317	76	60	136
120 Males Attracting						
1	38	23	61	3	3	6
2	240	619	859	4	6	10
3	10	23	33	4	6	10
4	40	29	69	1	1	2
5	36	40	76	6	9	15
6	254	435	689	4	10	14
Σ	618	1169	1787	22	35	57

Fly abundance in experiment 3 also showed a significant, though weaker, non-linear relationship to host abundance: adding a quadratic term for host abundance, r^2 improved from 16.9% to 27.8% ($F=6.76_{(1,16)}$, $p<0.01$).

Experiments 2 & 3: Sandfly Sex Ratio

The sex ratio of *Lu. longipalpis* caught on each shed-night was modelled in GLIM (NAG U.K. Ltd., Oxford) as the Ln Odds Ratio (Males:Females) with the Logit link function. The sex ratio in experiment 3 was significantly more male-biased than experiment 2 (0.61 vs. 0.51; $\chi^2_1=8.34$; $p<0.005$). In experiment 3, the sex ratio became more male-biased with increasing host abundance (estimate(s.e.) = 0.349(0.0418), $\chi^2_1=5.8$, $p<0.025$), but in experiment 2 this was only true within the marked sub-population (estimate(s.e.) = 0.025(0.036), $\chi^2_1 = 0.2$, $p>0.6$). No overall correlation was found in experiment 2 (estimate(s.e.)=0.143(0.034), $\chi^2_1=0.7$, $p>0.4$).

The inclusion of trapping night as a factor did not improve any of the models of sex ratio significantly, suggesting a behavioural rather than ecological explanation for the relationship between host abundance and sex ratio.

Experiment 2: Aggregation Dynamics Within Nights

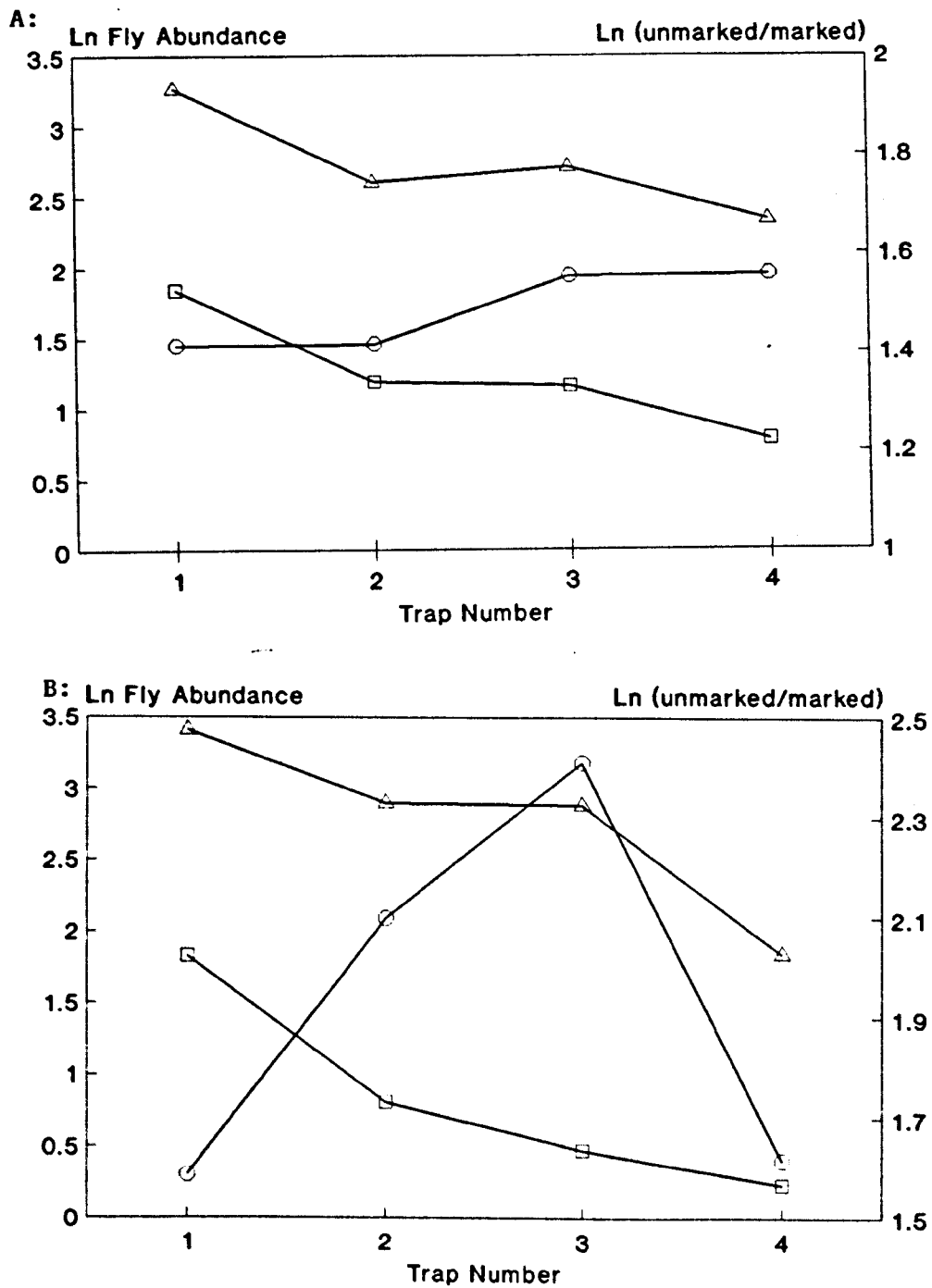
Migration to and from one shed. Twenty-one shed-nights were included in the analysis. The hourly catch rate in trap 4 (2400-0630 hours) was estimated by dividing by 6.5. Figure III.2 shows the geometric mean change in abundance of marked and unmarked flies, and their ratios, for all shed-nights. Table III.2 gives the estimates of the mean rate of change between each time step: traps 1-2 (2100-2200 hours); traps 2-3 (22.01-2300 hours) and traps 3-4 (24.01-0630

Table III.2. Regression estimates of the slope of the change in geometric mean unmarked and marked flies, and their ratio (unmarked:marked) between trap catches.

Trap Numbers	FEMALES		MALES	
	Estimate (S.E.)	t _{1,19}	Estimate (S.E.)	t _{1,19}
Marked				
1-2	-1.014 (0.355)	2.86 [*]	-0.652 (0.502)	1.3
2-3	-0.340 (0.269)	1.26	-0.034 (0.381)	0.09
3-4	-0.241 (0.198)	1.21	-0.381 (0.279)	1.37
1-2-3	-0.611 (0.117) ^a	5.22 ^{***}	-0.281 (0.068) ^a	4.13 ^{***}
Unmarked				
1-2	-0.511 (0.411)	1.24	-0.652 (0.582)	1.07
2-3	-0.021 (0.381)	0.06	0.099 (0.538)	0.18
3-4	-1.036 (0.328)	3.16 ^{**}	-0.377 (0.464)	0.81
Unmarked:Marked				
1-2	0.504 (0.307)	1.64	0.001 (0.434)	0.0
2-3	0.320 (0.279)	1.15	0.135 (0.394)	0.34
3-4	-0.795 (0.242) ^b	3.29 ^{**}	0.003 (0.342) ^b	0.01
1-2-3	0.394 (0.111) ^c	3.55 ^{***}	0.082 (0.065) ^c	1.26

*=p<0.01, **=p<0.005, ***=p<0.001; paired letters in superscript after (S.E.) indicate significant differences between estimates at 5% level.

Figure III.2. Changes in log abundance of marked (\square) and unmarked (Δ) flies and their ratio (marked:unmarked) (\circ) in sheds over traps 1-4 (2100-0630 hours). A = males; B = females.



hours), calculated by ANCOVA, with sex as a two-level factor, and trap number as a continuous variable.

The rate of decline of marked females (equivalent to the emigration rate) was highest, and significantly greater than zero, between traps 1 and 2 (Fig. III.2b). Thereafter it continued to fall with each successive trap, though not significantly. The abundance of marked males also declined consistently, though at a slower rate than females, and not significantly so between any one pair of traps (Fig. III.2a). Overall, between 2100 hours and midnight, the emigration rate of females was approximately twice that of males.

The abundance of unmarked males and females also fell consistently. The resulting estimates of unmarked:marked males (equivalent to the immigration rate) did not vary significantly over the trapping period, which is to say that immigration rates were close to zero. The ratio of unmarked:marked females increased between 2100 hours and midnight, giving an estimated immigration rate of 0.394 per hour (Table III.2).

Between midnight and 0630 hours, however, the female ratio fell significantly, which is not allowed in our model (Eq. III.7). The implication is that unmarked flies left faster than marked, sugar-fed flies, perhaps to search for a sugar meal elsewhere before dawn. In any event, the fall in unmarked:marked females indicates that female immigration had ended by 2400 hours.

We can also calculate the emigration and immigration rates between 2100 hours and midnight, and investigate the way they are influenced by fly and host abundance (Table III.3).

The emigration rate of males decreased as male and host abundance increased. Female abundance had no effect unless male abundance was excluded (estimate(s.e.) = -0.5112 (0.1579); $t_{1,20}=3.24$; $p<0.01$). The emigration rate of females tended to increase with the number of hosts, and decrease with the number of females. The number of males had no effect unless females

Table III.3. Correlates of the slope of the change in log marked flies (emigration rate) and the ratio of unmarked:marked (immigration rate) flies in individual sheds between trap catches 1-3 (2100-2400 hours).

Explanatory Variable	MALES		FEMALES	
	Estimate (S.E.)	t _{1,19}	Estimate (S.E.)	t _{1,19}
Emigration				
Ln Males	-0.6495 (0.1716)	3.78***	-0.1429 (0.1734)	0.82
Ln Females	-0.1102 (0.1726)	0.64	-0.5171 (0.1745)	2.96**
Ln Hosts	-0.4957 (0.2199)	2.25*	0.4384 (0.2308)	1.97
Immigration				
Ln Males	0.2739 (0.2151)	1.27	-0.1433 (0.1995)	0.72
Ln Females	-0.1417 (0.2164)	0.65	0.4338 (0.2007)	2.16*
Ln Host	-0.4858 (0.2762)	1.76	0.2360 (0.2560)	0.92

*=p<0.05, **=p<0.01, ***=p<0.005

were excluded (estimate(s.e.) = -0.4583 (0.15); $t_{1,20}=3.06$; $p<0.01$).

The immigration rate of females was higher when more females, or more males (estimate(s.e.) = 0.3539 (0.1648); $t_{1,20}=2.15$; $p<0.05$), were present, but hosts had no effect. None of the three variables explained significant variation in the immigration rate of males, consistent with the summary statistics (Fig. III.2a; Table III.2), which showed that there was no significant immigration of males over the trapping period.

Migration between two sheds. Eighteen shed-nights from nine nights were included in the analysis. The geometric mean number of marked males and females caught at away sheds were not significantly different (males (95% C.I.): 5.1 (3.75-6.94); females (95% C.I.): 3.84 (3.05-4.84)).

The rate of arrival of marked males at the away shed was inversely dependent on male and female abundance in the home shed, implying that, as male and female abundance increased, the emigration rate of males decreased, consistent with the results above (Table III.4). The rate of arrival of marked females at the away shed correlated positively with male abundance in the home shed, implying that the emigration rate of females increased with male density. If male and female estimates are treated as interchangeable, this result apparently contradicts the alternative analysis of female emigration. However, male abundance may be acting as an index of female abundance (substituting female abundance for male abundance: estimate(s.e.)= 1.159(0.0853)), and it is known that failure to take a full bloodmeal increases with female density (Chapter II). The positive correlation between male abundance and the emigration rate of females may therefore reflect an increasing rate of failure of females to obtain a bloodmeal in the home shed. These are the females which would be expected to arrive at the away shed, in search of a new host site rather than a resting site.

Table III.4. Correlates of the log total number of marked males and females migrating to the 'away' shed caught in traps 1-4 (2100-0630 hours) with abundance of males, females and hosts in the home (h) and away (a) sheds.

Outcome Variable	Explanatory Variable	Estimate (S.E.)	F _{d.f.}
Males			
	Ln Males _h	-0.3039 (0.0995)	5.02 _{1,13} *
	Ln Females _h	-0.3793 (0.1412)	5.39 _{1,13} *
	Ln Males _a	0.7862 (0.1827)	6.88 _{1,13} *
	Ln Hosts _a	0.3680 (0.0949)	6.61 _{1,13} *
Females			
	Ln Males _h	0.1347 (0.0430)	7.26 _{1,15} *
	Ln Males _a	0.2286 (0.0761)	7.03 _{1,15} *

*=p<0.05

The rate of arrival of marked males at the away shed is directly dependent on male and host abundance in the away shed, implying that the immigration rate of males increases with male and host abundance. This could not be estimated by the alternative analysis, which depended on immigration after 2100 hours. The effect of male abundance on immigration was significantly greater than that of host abundance ($t=3.35$, $p<0.01$). The rate of arrival of marked females at the away shed correlated positively with male abundance at the away shed, implying that the immigration rate of females increased with male abundance, agreeing with the results above.

Experiment 3: Aggregation Dynamics Between Nights

Compared with experiment 2, the geometric mean numbers of marked males (night 1 (95% C.I.): 1.52 (1.19-1.93); night 2: 1.51 (1.04-2.19)) and females (night 1: 1.18 (0.97-1.44); night 2: 1.13 (0.94-1.35)) caught at away sheds in experiment 3 were very low, reflecting the lower overall abundance of flies. As a result, no correlates were found of the movement of marked flies between sheds on the first and second nights of experiment 3. Nevertheless, marked flies joining aggregations on night 2 were clearly not distributing themselves randomly between home and away sheds. Capture of marked flies on night 2 was overwhelmingly at the home shed (92.7%), and the proportion of marked males returning from night 1 was significantly greater than females ($\chi^2_1=29$, $p<0.001$) (Table III.5). The proportion of marked males and females caught away on night 2 was greater than night 1, but the size of the confidence intervals suggest that this could have been achieved if all flies had returned to the same shed on night 2 and then emigrated to the away shed at the same rate as on night 1.

Site fidelity between nights may simply have been a result of the location of the day-time

resting sites: flies resting nearer to the home shed than the away shed were more likely to return to the home shed on night 2. Most flies do not rest inside sheds (Quinnell and Dye 1994a), but must travel at least a short distance outside the shed to rest during the day. Therefore a shed at zero meters from the home shed should, theoretically, attract half the flies returning on night 2. In this case, a regression model of distance between home and away sheds versus the proportion of marked flies caught away on night 2 should have an intercept of 0.5 at zero meters. The ratio of marked flies (male+female) at home:away sheds was modelled in GLIM with the logit link function, and regressed against the distance between home and away sheds. The slope of the best fit index of distance (distance untransformed) was negative, as expected, but not significantly different from zero ($F_{1,14}=2$; $p>0.05$). The intercept of 0.24 was, however, significantly different from 0.5 (95% CI: 0.111-0.4435), suggesting that something more than a resting site bias is involved in site fidelity of flies between nights 1 and 2.

Table III.5. The geometric mean proportion of flies caught in home sheds (h) that are marked (C) and the geometric mean proportion of marked flies that are caught at away sheds (a) on nights 1 (N1) and 2 (N2), and the geometric mean proportion of marked flies at the home shed that are caught on night 2.

			Geometric Mean (95% C.I.)
Measure			
Night 1	♂	$C_h / (C_h + U_h)$	0.305 (0.284-0.327)
		$C_a / (C_h + C_a)$	0.008 (0.004-0.017)
	♀	$C_h / (C_h + U_h)$	0.245 (0.217-0.266)
		$C_a / (C_h + C_a)$	0.012 (0.004-0.034)
Night 2	♂	$C_h / (C_h + U_h)$	0.088 (0.076-0.102)
		$C_a / (C_h + C_a)$	0.073 (0.042-0.122)
	♀	$C_h / (C_h + U_h)$	0.012 (0.007-0.02)
		$C_a / (C_h + C_a)$	0.158 (0.004-0.438)
Night 1-2	♂	$C_h N2 / (C_h N1 + C_h N2)$	0.237 (0.207-0.268)
	♀	$C_h N2 / (C_h N1 + C_h N2)$	0.046 (0.029-0.077)

DISCUSSION

Aggregation Dynamics Within Nights

Based on the results of experiments 1, 2 and 3, we propose the following scheme for the aggregation dynamics of *Lu.longipalpis*.

Males were on the wing earlier than females, as demonstrated by the early-evening sex ratio. However, by 2100 hours, detectable male immigration had ceased (Fig. III.2a, Table III.2). The immigration rate of males prior to 2100 hours increased with both host and fly abundance, though fly abundance had a considerably greater influence (Table III.4). Female immigration began later, and was detectable up to midnight (Fig. III.2b, Table III.2). It is possible that some females were delayed in searching for a feeding site by the activities of oviposition, as is true of other bloodsucking diptera. However, by searching for a host site when male aggregations have become established, females should also maximise the benefit of pheromone production for aggregation location. Unlike males, the immigration rate of females only increased with fly abundance (Tables III.3 & III.4). The fact that the sex ratio in experiment 3 became more male-biased with increasing host abundance is also suggestive of a difference in response of males and females to host kairomones.

Overall, females emigrated more rapidly than males (Fig. III.2a, Fig. III.2b, Table III.2), reflecting the different imperatives of the two sexes: males to maximise mating success, females to feed, mate and then seek a resting site. The emigration rate of males was inversely related to host and fly abundance, suggesting that males were using semiochemicals to maintain their position at the aggregation (Table III.3). In contrast, the emigration rate of females seems to have been determined by the logistics of blood-feeding, rather than by semiochemicals. The

female emigration rate decreased with fly abundance, but increased with host abundance. It is known that increasing densities of females at the host lead to a reduction in per capita feeding success, which is partially relieved by increasing host abundance (Chapter II). The differential influence of host abundance on the emigration rate of males and females is further implicated by the fact that only the sex ratio of marked flies was sensitive to host abundance in experiment 2.

The spatial scale of experiment 2 was considerably restricted, compared with the distances commonly recorded for *Lu.longipalpis* movement, and all sheds were similarly-constructed, suggesting a behavioural explanation (rather than an ecological one) for the association of host and fly abundance with immigration and emigration rates. The obvious explanation is that the former were directly effecting the latter, through the medium of semiochemical production. This is most plausible of host abundance; it is more difficult to imply causality between fly abundance and immigration and emigration, since where the immigration rate is higher and the emigration rate is lower there will be greater fly abundance.

However, the effect of the male bait in experiment 1 illustrates again the dramatic effect of male pheromone on aggregation formation (Table III.1) (see also Dye *et al* 1991). The female bait also produced an increase in aggregation size, though the effect was weaker (Table III.1). Females are not known to produce a pheromone, but they can communicate with males at close range visually and by wing beating (Ward *et al* 1988). Males arrested in this way by females would then attract more males and females.

Strong circumstantial evidence for a causal link between fly abundance and immigration and emigration rates is provided by the distribution of males and females across sheds of different host abundance (Fig. III.1). Increasing fly abundance with host abundance may be explained by the early influence of host abundance on the immigration rate of males. Throughout

the night, however, host abundance, and therefore kairomone production, remains constant. The non-linearity in the relationship between host and fly abundance must therefore result from changes in the level of pheromone production as fly abundance increases. The first males to arrive in the evening, in response to host factors, prime aggregation formation, producing pheromone and attracting and arresting more flies, which in turn attract and arrest yet more flies. In this way, sites which first attract and arrest more flies as a result of greater host abundance will increasingly dominate recruitment, resulting in the observed non-linear distribution.

Aggregation Dynamics Between Nights

Flies returned preferentially to the site of their previous night's aggregation (Table III.5). This will also prime aggregation formation, favouring those sites which had larger aggregations the night before. More males than females returned on night 2, presumably because females are occupied with digestion at the resting site and breeding site location. This provides an alternative explanation of the change in sex ratio observed by Dye *et al* (1991) during the colonisation of new sheds. Females, uncommitted to a particular aggregation on their return from the breeding site, would predominate in new sheds. This is exemplified by the greater overall female bias in sex ratio from experiment 1, compared with experiment 3. The male bias would then increase over the following nights, as recruitment increased and more males than females returned on subsequent nights.

The degree of site fidelity was greater than could be adequately explained by a resting site bias. One possible explanation is that flies 'remember' the location of an aggregation site. Genes linked to visual and olfactory learning have been identified in cyclorrhaphan diptera (e.g. Folkers 1982; Kyriacou & Hall 1984). Work on blood-sucking nematocerans has also raised the

possibility that they are capable of learning a "familiar area map" (Baker 1982; Charlwood *et al* 1988; Renshaw *et al* 1994), presumably to facilitate movement between feeding, resting and breeding sites. Such a system would clearly reduce the costs of host-searching by *Lu.longipalpis*, assuming hosts habitually passed the night at the same site.

Pseudoreplication in the Analysis

The analysis is open to the criticism that repeat catches in sheds are not independent, and therefore pseudoreplicates. We argue that the data are independent for the variables under investigation, that is fly and host density. By attempting to correct for supposed pseudoreplication in the usual manner - averaging host and fly abundance over all the repeats for each shed - we are averaging away the variation in which we are interested. However, by not doing so, the analysis is open to one important criticism. Males return preferentially to the same shed, night on night, therefore for that proportion of male immigrants which are returning from the previous night (23.7%) (Table III.5) immigration rate is not independent of shed location. However, we believe that this affects only the analysis of male immigration rate in the migration to and from one shed in Experiment 2, which is not crucial to our interpretation of the data. Females are not biased towards shed on the basis of position. Secondly, the decision to leave one shed for another on the same night (migration between two sheds) should not be affected by male site fidelity between nights.

Implications for the Control of Lutzomyia longipalpis

The present study offers alternative explanations for the observations of Dye *et al* (1991):

we interpret the increasing male bias with host abundance as the result of a difference between sexes in emigration rate, rather than immigration rate; we interpret the increasing male bias with length of establishment of aggregation site (and therefore fly abundance) as resulting from a difference between the sexes in site fidelity, rather than immigration rates. We find no evidence that females in the peridomestic arena are being recruited to host sites at close to the maximum rate, as Dye *et al* (1991) suggest, and therefore no evidence that pheromone-baited traps could not succeed in increasing recruitment of females to a trap.

The targets of residual insecticide spraying regimes against *Lu.longipalpis* typically include animal pens (see review in Chapter IV), which contain most available hosts and which are therefore the main foci of fly activity. However, given the dominance of fly abundance in aggregation formation (Table III.4), it is possible that the disruption of pheromone production by spraying would displace the fly population to the remaining pheromone sources at unsprayed sites. A corollary of the non-linear relationship between host and fly abundance is that most host sites are relatively under-exploited by females. It is therefore doubtful whether female feeding success would be significantly reduced, and the sandfly population diminished. Finally, by displacing fly populations from non-competent hosts such as chickens, spraying animal pens could elevate the biting rate on canids and humans, increasing the transmission of American Visceral Leishmaniasis. Future residual spraying regimes at host sites might therefore benefit from application of a synthetic male pheromone, together with the insecticide, in order to maintain fly recruitment.

CHAPTER IV

A SHED-LEVEL INTERVENTION TRIAL FOR THE CONTROL OF PERIDOMESTIC POPULATIONS OF *Lutzomyia longipalpis*

SUMMARY

- (1) Using the insecticide lambda-cyhalothrin ("Icon"), an intervention trial was conducted to study the effect of focal and blanket coverage of animal pens, the major aggregation sites of peridomestic *Lutzomyia longipalpis*, on fly abundance and distribution.
- (2) A 90% reduction in abundance was achieved in sheds of the focal intervention, where single sheds were treated. However, there was no discernable effect on abundance of other phlebotomines in sheds, or the abundance of *Lu.longipalpis* in dining-huts and houses.
- (3) As the mortality rate of *Lu.longipalpis* and the other phlebotomines was similar, the differential reduction in *Lu.longipalpis* abundance is interpreted as a reduction in recruitment of *Lu.longipalpis* to the treated site due to insecticide-mediated male death and consequent reduction in pheromone production. This assumes that few, if any of the other phlebotomines caught used pheromone to attract conspecifics to sheds.
- (4) Our explanation is supported by evidence of (i) a disproportionate reduction in male abundance over female, and (ii) a smaller effect on abundance of gravid females, which are not attracted to pheromone, over other gonotrophic classes.
- (5) At the blanket intervention site, abundance of *Lu.longipalpis* only fell by 50%. We argue that because all major aggregation sites at the site were sprayed, there was less change in the relative attractiveness of treated sheds, and thus less reduction in recruitment.
- (6) Here, catches at untreated dining-huts increased in size. We argue that this is a result of the reduction in attractiveness of all the major neighbouring sites.
- (7) It is recommended that care be taken during blanket intervention programmes to ensure that *all* potential aggregation sites are treated. The possible consequences of leaving some sites untreated are poor control of peridomestic sandfly abundance and possibly an increase in the biting rate on dogs and humans.
- (8) A comparison of insecticide-impregnated target sheets versus residual spraying showed residual spraying was approximately twice as effective as target-sheets in reducing fly abundance.

INTRODUCTION

The Impact of DDT House-Spraying on Leishmaniasis

In Brazil, as in the rest of the world, control of the phlebotomine vectors of leishmaniasis has, for the most part, been incidental to anti-malaria campaigns. These commonly involve the spraying of residual insecticides, principally DDT, within houses. The best evidence for the efficacy of house spraying against leishmaniasis often comes on cessation of the malaria control effort. In Iran, for example, Sayedi-Rashti & Nadina (1975) report the re-emergence of cutaneous leishmaniasis at the end of a DDT anti-malarial campaign; Mukhopadhyay *et al* (1987) observed a resurgence of *Phlebotomus argentipes* and *P. papatasi* in Bihar, India after DDT spraying.

A reduction in the incidence of leishmaniasis during anti-malarial DDT house spraying has been reported in Iran (Nadim & Amini, 1970), and Pakistan, Israel and Tunisia (reviewed in Turner, 1965). However, anti-malarial programmes are not always associated with a reduction in disease incidence, particularly where vectors are exophagic. For example, in the plains region of China, *L. donovani*, agent of kala-azar, is transmitted by two vectors: *P. chinensis* (endophilic) and *P. chinensis longiductus* ('peridomestic'). Through a long and sustained program of DDT and BHC residual house spraying, begun in the 1950s, field surveys have failed to detect *P. chinensis*, and no new cases of VL have occurred for many years in those areas. However, where the less endophilic *P. chinensis longiductus* is present, new cases of kala-azar continue to be reported (Guan, 1991).

In our study area, DDT house spraying against malaria vectors is associated with a reduction in the abundance of *Lu. longipalpis* within houses. Quinnell and Dye (1994b) found

populations 50% lower in houses 12 months after spraying, and 25% lower after 24 months; Lane (1991) refers to unpublished work by Santos, elsewhere in Brazil, which records pre-spray levels of sandflies six months after a single application of DDT. However, *Lu.longipalpis* is facultatively exophagic in that its ability to access houses is limited: the more open a structure, the more flies are able to enter, forming pheromone-producing leks and attracting yet more flies and competing more effectively for flies with other leks (Quinnell & Dye 1994a). In this way fly populations become polarised away from well-constructed houses, principally to animal pens. Predictably then, house spraying has no discernible impact on the sandfly population outside houses.

It is not clear whether residual house spraying alone has sufficient impact on the infective man-biting rate. Since the *Lu.longipalpis* population without the house remains high, the parasite reservoir in dogs, which commonly sleep outside, will be unchanged and the number of infected sandflies will therefore also be unaltered. In this case, transmission to humans will be more or less undiminished, depending on the proportion of infective bites which are received by humans outside the house.

The Impact of Outdoor Spraying on Lutzomyia longipalpis

Unlike malaria control programmes, interventions specifically targeted against *Lu.longipalpis* and AVL involve the spraying of both houses and animal shelters, in an attempt to reduce the outdoor fly population. Unfortunately, most of the published data report on the retrospective analysis of *ad hoc* responses to epidemics. No entomological measures are reported, and assessment of the impact of vector control (indoor and/or outdoor) on disease transmission is therefore complicated by the typically integrated nature of the approach -

insecticide spraying, dog elimination and human case treatment - and lack of controls.

The following is a brief review of the evidence for the control of disease transmission through the control of peridomestic populations of *Lu.longipalpis* within integrated control programs:

(i) In Minas Gerais, S.E. Brazil, in a control program spanning 15 years (1965-79), there was blanket coverage of houses and animal pens with DDT, seropositive dogs and seronegative dogs showing any clinical symptoms suggestive of *L.chagasi* were destroyed, and all human cases were treated. Human cases disappeared by 1978 (Magalhães , 1980).

(ii) In Rio de Janeiro, Nunes *et al* (1991) presented data for a fall in the percentage of dogs seropositive by IFAT coincidental with a large-scale yearly DDT spraying program covering all houses and sheds within 100m of houses having seropositive dogs, together with the elimination of all seropositive dogs.

(iii) In the city of Teresina, and the surrounding rural areas of Piauí State, North-Eastern Brazil, an epidemic of AVL occurred between 1980 and 1986. In Teresina, vector control through blanket coverage of houses began in 1981 with BHC house-spraying, then changed to DDT, and finally street fogging with the organophosphate 'Sumithion'. Contemporaneous with this, seropositive dogs were eliminated. Outside Teresina, the highest incidence of AVL occurred in the North of the state, where anti-malaria DDT house spraying occurred, though generally with coverage of less than 10% of houses. Thus the rural epidemic can be treated as a control for the effect of insecticide spraying and fogging against incidence of AVL. Commencement of the intervention coincided with a reduction in disease incidence in Teresina. At the same time, however, the epidemic ended spontaneously in rural areas, and analysis of the changing incidence of disease between age groups in Teresina suggests that the epidemic there may have ended naturally, as a result of a reduction in susceptible infants (Costa *et al*, 1990).

(iv) In Ceará, N.E. Brazil, Alencar (1961) reported on a control regime from 1953 to 1960. All seropositive dogs and foxes (*Lycalopex vetulus*) and seronegatives with suspicious symptomology were destroyed, and human cases were treated. Most interestingly, DDT spraying (houses and animal pens) was only conducted in one of the two groups of 14 counties within the state, all of which were subject to the other control measures. Spraying commenced on average in 1956-7 in the intervention group and the number of human cases fell by 58% between 1953-6 (765 cases) and 1957-60 (320 cases)(paired t-test for the difference: $0.01 > p > 0.001$). In the 14 counties not receiving DDT, cases rose by 12% between 1953-1956 (89 cases) and 1957-60 (101 cases)($0.6 > p > 0.5$)(two sample t-test, unsprayed vs. sprayed: $p = 0.0011$). These data must be treated with some caution: there is no denominator population given, so the data do not represent incidence. Furthermore, counties in the unsprayed group have a much lower mean number of cases in the first period (6.4, s.d.=3.2) compared with the sprayed group (54.6, s.d.=39.3). Thus questions are raised about the homogeneity of counties assigned to each treatment group.

In summary, the above studies provide limited evidence that DDT spraying is important to the control of AVL within an integrated control programme. However, there is no clue to which of the elements of the spraying regime - indoor or outdoor, focal or blanket - are important, or whether vector control alone is sufficient.

In the only study specifically to investigate the impact of insecticide spraying on peridomestic *Lu.longipalpis* populations, Le Pont *et al* (1989) reported on an intervention trial spraying the synthetic pyrethroid deltamethrin inside and outside houses in a focus of AVL in Bolivia.

Two months' pre-treatment data were recorded in 50 houses and 7 chicken sheds from the Andean village of Los Yungas. The following month, deltamethrin was applied at a

concentration of 0.025gm^{-2} to the entire interior surface and exterior walls of houses, their dining shelters, chicken sheds, dog kennels, piles of adobe and the trunks of nearby trees. It was estimated that for each domestic unit, a minimum 46m^2 were sprayed. All 70 houses in the village, and their environs, were sprayed, and a comparison of the month pre-treatment versus 11 months post-treatment was made.

Throughout the peridomestic environment, the two most commonly captured species of sandfly were *Lu.longipalpis* and *Lu. nuneztovari anglesi*, the presumed vector of cutaneous leishmaniasis in the area. Post-intervention, the average number of female *Lu.longipalpis* per house trap fell from 0.7 to zero in three months, and remained negative for five of the seven months following, when densities of 1.2 and 1.4 females per house were recorded, on the tenth and eleventh months post-treatment. In chicken sheds, females per shed trap fell from 40.2 to zero in one month post-treatment, and remained that way until the eleventh month when, dramatically, a catch of 214 females per shed was made. By contrast, the density of *Lu.n.anglesi*, which started at 10.78 females per house and 2.3 per chicken shed, never reached zero, and was erratically higher or lower than pre-treatment in the months following treatment. This, it is suggested, may be a result of the greater exophilicity of this fly.

The study seems to demonstrate that mass-killing of *Lu.longipalpis* is possible, but the design is not ideal. The numbers caught were very low compared with typical fly abundance in our own and many other Brazilian sites. There is also no untreated village to provide a contemporaneous control for seasonal variations, though Le Pont & Desjeux (1985) report that *Lu.longipalpis* is usually abundant throughout the year.

More seriously, because all post-treatment trapping is conducted at the sprayed sites, there is no independent assessment of fly abundance in the village. The results do not, therefore, preclude the possibility that *Lu.longipalpis* are being attracted away from the treated areas to

unsprayed sites. Yet this is the possibility raised by the results of the preceding chapters: by disrupting production of male pheromone - the major aggregation semiochemical (see Chapter III) - through death or repellency of males, insecticide spraying may cause remaining flies to be preferentially attracted to the remaining unsprayed host sites with little cost to feeding success (see Chapter II), and thus with no mass-killing of the fly population.

The primary aim of the present study is therefore to investigate the effect of treating animal pens with insecticide on peridomestic fly abundance and distribution.

New Methods in Sandfly Control

A secondary aim of the present study is to test a new method of delivering insecticide to the control site. New insecticides, principally the synthetic pyrethroids, are increasingly popular in vector control projects. Per unit of active ingredient, they are far more expensive than other classes of insecticide, but they have lower LD50s and so are applied at proportionately lower concentrations. Coupled with novel delivery systems, which limit the surface area that need be sprayed, vector control with pyrethroids is becoming cost-competitive with traditional DDT spraying methods.

Since the mid-80s, permethrin-impregnated curtains have been used in trials for the control of malaria vectors (Lines , 1985), and later against sandflies in Italy (Maroli & Lane, 1987) and Burkina Faso (Majori , 1989). In the latter study, permethrin-impregnated nets (1gm^{-2}) were hung inside doorways and around the eaves of houses and approximately 99% control of indoor phlebotomines was recorded. In contrast to the use of impregnated nets as barriers, Mutinga *et al* (1992) conducted a control trial in six villages (2000 houses) in Kenya using cloths as targets. Cloths, made of cotton netting treated with 0.5gm^{-2} permethrin and measuring $1.5 \times$

9m, were hung on the wall inside houses. Most houses had only a single room. Compared with control villages, treated houses showed a reduction of 76% for *Phlebotomus martini*, vector of visceral leishmaniasis, and 85% for *Phlebotomus duboscqi*, vector of cutaneous leishmaniasis, compared with pre-spraying levels. However, sandfly populations in houses in two control villages also fall, by approximately 40%. If a real reduction did result from the installation of wall cloths, the authors argue that it is due to delivery of a lethal dose to sandflies contacting the cloth as they hop on the walls prior to feeding. This they conclude from data showing a trapping-out of sandfly populations in houses when using 1m² sticky traps on walls (Mutinga, 1981).

From the sugar-marking work in Chapter III, it was noted that approximately 40% of males and females caught in CDC traps run concurrently in the same shed were marked. This can be taken as the minimum percentage contacting the sheet, and it is proposed that the sheet acts as a target, providing a convenient resting site for flies before entering the lek. By impregnating such targets with insecticide, it might be possible to achieve control in a manner similar to mbu wall cloths. The advantage over residual spraying is that it considerably reduces the surface area to be sprayed, and allows convenient and standardised preparation at a central location for rapid distribution.

MATERIALS & METHODS

Study Area and Timing

Houses were normally of mud floors and walls with *najá* palm roofs; homesteads were usually planted with fruiting trees, and domestic animals - dogs, pigs and fowl - were common. Fowl were housed in sheds comprising a close palisade of wooden stakes and a roof of *najá* palm. Where there was no shed, fowl usually roosted in trees.

In addition to houses and animal pens, many homesteads contained dining-huts. These were open-sided structures, with a *najá* palm-thatch roof where meals were cooked and eaten and members of the household socialised. Thus dining-huts were typically occupied at night until bedtime (approximately 21.00hrs).

Seven villages were included in the study: Campinas (CA, 36 houses), Pingo d'Agua (PA, 32 houses), Estrada (ES, 15 houses), Vila Ceará (VC, 8 houses), Vila da França (VF, 10 houses), Vila Nova (VN, 8 houses) and Bacabau (BA, 27 houses), each separated from the nearest neighbouring village by 0.5-2km as the crow (or sandfly?) flies.

Two pre-treatment trapping rounds were conducted from October 16 1993 to November 11 of the same year, towards the end of the dry season in Northern Brazil. Meteorological conditions in the area were relatively constant over this first sampling period, with an average temperature of 27.9°C (sd. 0.27)(@21.00hrs), relative humidity of 79.1% (sd. 2.79)(@21.00hrs) and daily precipitation of 0.1mm (sd. 0.29)(data from the National Institute of Meteorology, MAARA, Belém, Brazil).

Continuous post-intervention sampling (rounds 3-7) began on November 22 1993 and ended on February 21 1994. A final trapping round was conducted from June 8-10 1994 (round

8). During the post-intervention period, the climate changed from dry to wet season. From late December (the beginning of round five), the meteorological conditions changed markedly, and became more variable. The average temperature fell to 26.8°C (sd. 1.244)(@21.00hrs), mean relative humidity was 85.2% (sd. 6.79)(@21.00hrs) and mean daily precipitation was 13.8mm (sd. 26.42).

Insecticide Regime

We used Lambda-Cyhalothrin 10% Microencapsulated ("Icon 10 ME"), at a dose of 20mg of active ingredient per square meter. Two treatments were used: at spray-treatment homesteads, a 10 litre Hudson sprayer was used to treat the inside and outside of the walls and roof of the chicken shed; at the target-treatment homesteads, a one meter square white cotton sheet, coated at the target concentration, was hung in the chicken shed approximately one meter from the roosting site.

Study Design

The study was divided into two qualitatively different interventions: focal and blanket coverage. For the focal spraying programme, thirty homesteads from within CA, PA, ES, VC, VN & BA were selected which had a chicken shed and were separated from the nearest next selected homestead by at least one other house. Nearest neighbours were then grouped into threes to form the subject of one night's trapping, totalling ten triplets on ten trapping nights. At the end of the two pre-treatment rounds, each chicken shed of a triplet was assigned to one of three treatment groups: spray, target or control (no insecticide), and for each triplet the

appropriate intervention was carried out on the morning before the first trapping night post-intervention. In this way each triplet was trapped at the same elapsed time post-intervention.

For the blanket spraying programme, trapping was conducted at all six of the homesteads in VF which had a chicken shed, and on the morning of the first post-intervention trapping night, all chicken sheds and other animal pens (three pig sties) in the village were sprayed. This left just one large group of peridomestic animals untreated: five adult chickens which habitually roosted in a fruit tree. A single chicken shed containing three chickens was left unsprayed as a control. Unfortunately, the family left the village shortly after intervention (for unconnected reasons!), and the control was therefore lost to the trial.

Entomological Measures

In every case, CDC miniature light-suction traps were set between 17.30hrs and 18.00hrs and collected between 06.15hrs and 06.45hrs. In each homestead, three traps were set in the house, usually one in each of the three rooms typically present, a single trap was set in the chicken shed, and where a dining-hut was present, a trap was also set above the dining table. Traps were always set in the same position, and as far as possible, the same trap was used at the same site for each trapping round in order to minimise the effect of variation in trap efficiency.

Catches were removed from traps by pooter as soon as possible, and normally before 11.00hrs. Live flies from chicken shed traps were removed first, into separate pooters, normally before 09.00hrs. The ratio of live:dead flies was then calculated as an index of insecticide activity. Flies were counted as male or female, *Lu.longipalpis* or "other", as described in Dye *et al* (1991). Female *Lu.longipalpis* were separated into four gonotrophic states - unfed, new fed, old fed and gravid - as described in Chapter II. Males of other phlebotomine species were

identified to species according to Ryan (1986), but pressure of work prevented the more time-consuming identification of their females.

Target-Sheet Removal

On rounds 6 and 7, target-sheets were removed from their sheds on the night following normal trapping, and a CDC trap was set in the usual way. Any flies caught were separated into live and dead and the ratio of live:dead, with and without the target-sheet, was used to provide a second estimate of the activity of the insecticide.

Analysis of the Focal Intervention

Essentially, the analysis involves a comparison of sandfly abundance at control sites versus treated sites (sprayed or with target-sheets).

Rather than making the conventional comparison of the absolute difference between two measures of fly abundance (Molineux *et al* 1976), we calculate the ratio, control: treatment. This overcomes the effect of falling abundance of flies in traps from round 4 onwards - the onset of the rainy season (Fig. IV.1) - which increasingly restricts the possible magnitude of the absolute difference between observed and expected abundance. The ratio of control:treatment is conveniently modelled in GLIM as the log odds of finding a fly at the treated site rather than the control site, with binomial errors and the LOGIT link function.

The pre-treatment relationship in abundance, say between a control shed and a sprayed shed, can be used as a predictor of the post-treatment relationship. How the true post-treatment relationship between control and treated sheds varies from that predicted can therefore be used

as a measure of the effect of treatment on fly abundance.

In the same way, comparisons can be made of fly abundance between the dining-huts or houses from control and treated homesteads. Our definition of a fly in the analysis can also be varied to mean *Lu.longipalpis*, other phlebotomines or females of a particular gonotrophic state. Furthermore, rather than comparing treatment:control, we can compare *Lu.longipalpis*: Other Phlebotomine Species, or Gravid:Other Gonotrophic states and so on.

The expected relationship between a control and a treated site is estimated from the ratio of the geometric mean abundance from the two pre-treatment trapping rounds. This is calculated separately for each trapping night, thus controlling for spatial and temporal variations in fly abundance. Next, the mean ratio of fly abundance in control:treatment is calculated for each trapping round post-treatment. The significance of the difference between the pre-treatment ratio and post-treatment ratio is then tested by ANCOVA.

For ease of comparison between treatments, in Figures IV.2-IV.6 changes in abundance are normalised by setting the mean of the pre-treatment ratios to one. The scale of the y-axis is logarithmic, and the slope of the lines therefore represent the relative magnitude of the changes between trapping rounds.

Finally, the mean fly abundance from the ten sites in each treatment over all trapping rounds is also used to calculate the overall ratio, treatment:control, over the entire post-treatment period (5-6 rounds, 12-29 weeks).

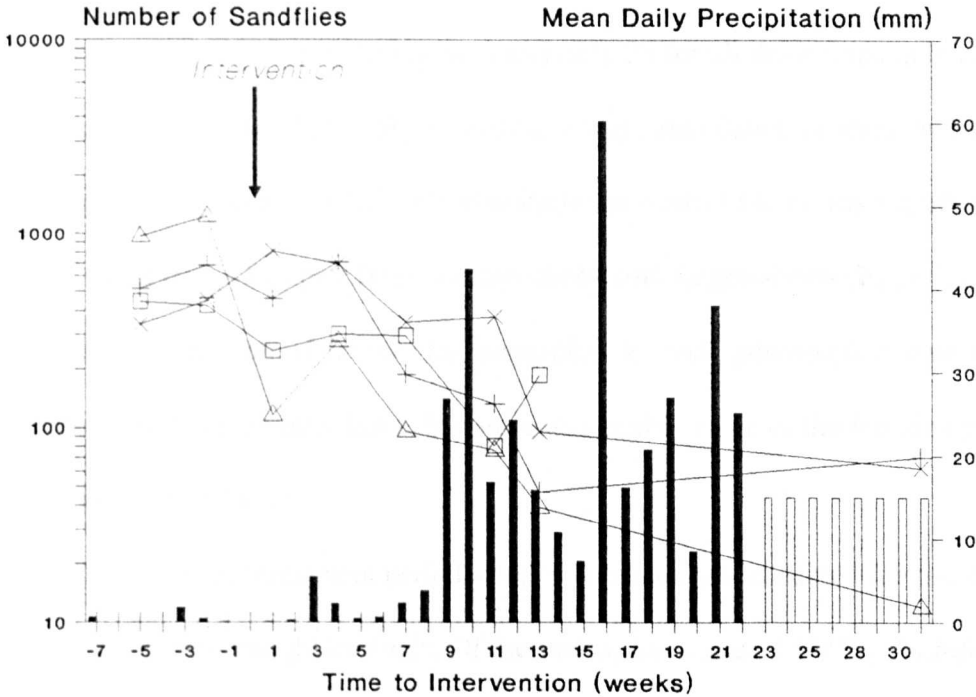
Analysis of the Blanket Intervention

Unlike the focal intervention, the blanket intervention does not have control sheds in the same village or trapped on the same night as the treated sheds. Instead, the mean abundance at

the corresponding site in the control homestead of the focal intervention is calculated for each trapping round, to estimate the expected abundance at each treated homestead in the blanket intervention.

This is not the ideal control, and the resulting analysis should be treated with caution. However, pre-treatment catches from the control sheds of the focal intervention and the treatment sheds from the blanket intervention are very similar both in terms of abundance of *Lu.longipalpis* and the species composition of other phlebotomines caught. This suggests that their ecologies are also very similar. Furthermore, the site of the blanket intervention (VF) lies both in the geographical centre of the study area and in the middle of the order in which each trapping round was conducted. This goes some way to controlling for spatial and temporal variation in catches between controls and treated sheds.

Figure IV.1. Changes over time in the abundance of *Lu.longipalpis* (males+females) in control sheds (x), sheds with target-sheets (+), sprayed sheds (Δ) and VF sheds (\square), and daily mean precipitation (filled bars). Empty bars = data not available.



RESULTS

Focal Intervention

Pre-treatment data. The geometric mean abundance of *Lu.longipalpis* in sheds was 658.5, compared with 77.3 in dining-huts and only 25 for all three traps in houses combined, a ratio of approximately 26:3:1. By treatment group, abundance in sheds was: control, 404.6; target-sheet, 622.3; spray, 1132.3. Fly abundance in control sheds was significantly lower than sprayed sheds ($F_{1,58}=7.2$, $p<0.001$), but not sheds with target-sheets ($F_{1,58}=1.4$, $p>0.05$).

The proportion of female *Lu.longipalpis* in each gonotrophic state in sheds closely resembled that in dining-huts, but in houses considerably more of the females caught are gravid and old fed (Table IV.1).

During the pre-treatment period, other phlebotomines accounted for less than 1% of male and female sandflies caught in sheds. Of the males, *Lu.evandroi* (74%), *Lu.infraspinosa* (11%) and *Lu.micropyga* (8%) were most commonly encountered. The total number and species of male phlebotomines caught from all trapping sites over the whole trial are shown in Table IV.2.

Chicken sheds. On day one post-treatment, the abundance of *Lu.longipalpis* (males + females) in sprayed sheds fell significantly to approximately 10% of that expected, and remained so until measurements ceased at round 8 (week 29) (Fig. IV.2a, Table IV.3). Abundance in sheds with target-sheets was only approximately 50% below the expected on day one. This fell to approximately 80% below the expected by round 7 (week 12 post-intervention), the only round on which abundance was significantly lower from the expected (Fig. IV.2a, Table IV.3). Overall abundance in sprayed sheds fell significantly more than in

Figure IV.2. Changes over time in the abundance of *Lu.longipalpis* (solid line) and other phlebotomines (broken line) at spray homesteads (Δ) and target-sheet homesteads (+), relative to control homesteads (set to 1). Shed = a; dining-hut = b; house = c.

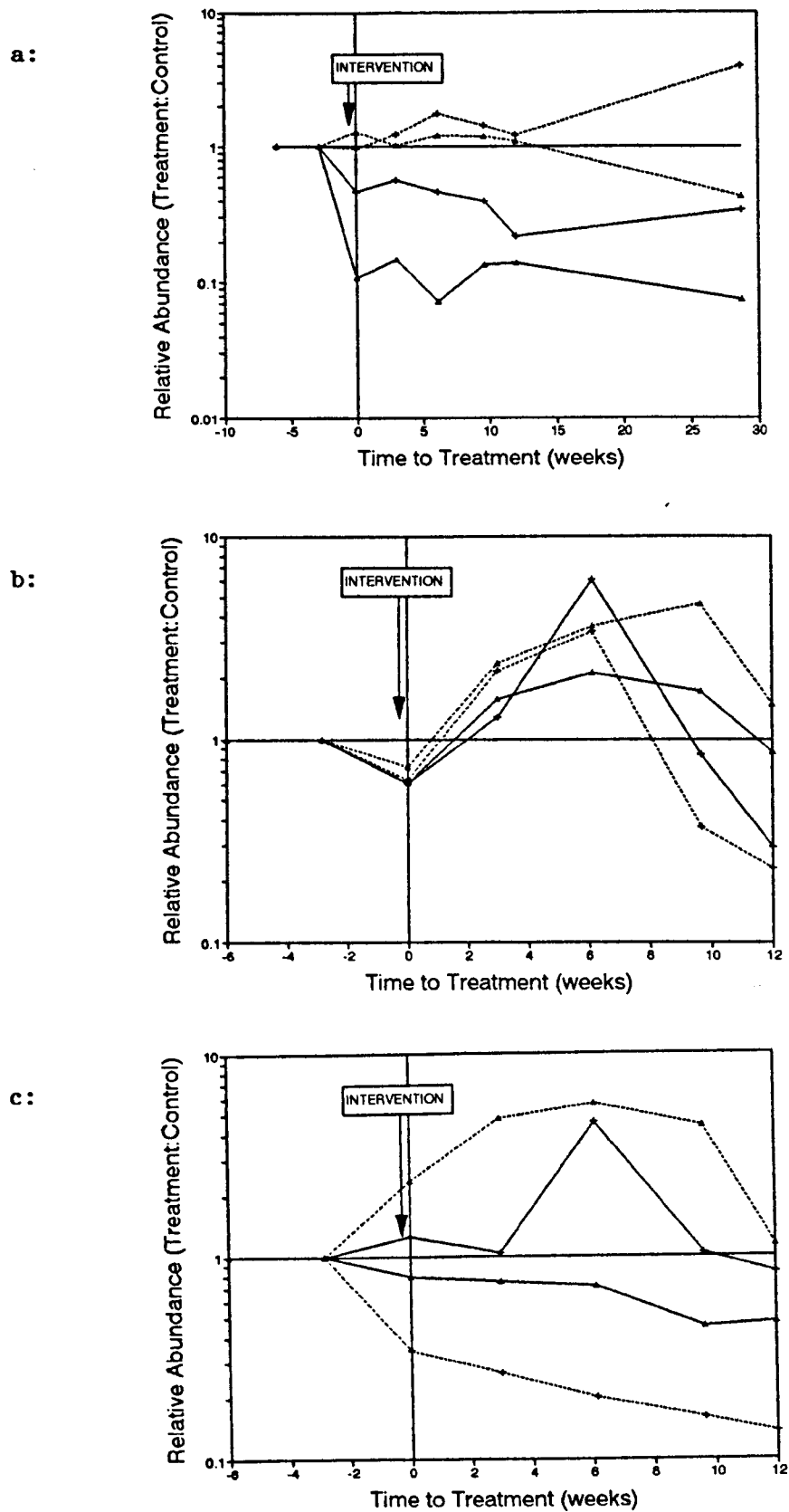


Table IV.1: The proportion of females trapped in sheds, dining-huts and houses during the pre-treatment period in the four gonotrophic states: unfed, new fed, old fed and gravid.

GONOTROPHIC STATUS	SHEDS	DINING-HUTS	HOUSES
Unfed	0.82	0.76	0.53
New Fed	0.09	0.08	0.07
Old Fed	0.07	0.07	0.13
Gravid	0.03	0.09	0.27

Table IV.2. Genus, sub-genus, species and number of male phlebotominae, other than *Lutzomyia longipalpis*, caught in CDC traps from all locations over trapping rounds 1-8.

SPECIES	ABUNDANCE
<i>Brumptomyia brumpti</i>	16
<i>B.travassosi</i>	4
<i>Lutzomyia aragaoi brasiliensis</i>	54
<i>Lu. aragaoi inflata</i>	1
<i>Lu. cayennensis micropyga</i>	75
<i>Lu. evandromyia infraspinosa</i>	216
<i>Lu. evandromyia monstrosa</i>	2
<i>Lu. lutzomyia longipalpis</i>	95292
<i>Lu. lutzomyia servulolimai</i>	7
<i>Lu. migonei evandroi</i>	1775
<i>Lu. nyssomyia antunesi</i>	14
<i>Lu. nyssomyia flaviscutelata</i>	35
<i>Lu. oswaldoi longipennis</i>	4
<i>Lu. oswaldoi trinidadensis</i>	115
<i>Lu. pilosa pilosa</i>	2
<i>Lu. trichophoromyia brachypyga</i>	24
<i>Lu. trichophoromyia ubiquitalis</i>	1
<i>Lu. viannamyia furcata</i>	8
<i>Psychodopygus geniculatus geniculatus</i>	1

Table IV.3. Changes in the ln Odds Ratio (treatment:control) of *Lu.longipalpis* abundance in sheds for target and spray sheds.

ROUND	TARGET (n=10)			SPRAY (n=10)		
	RATIO	χ^2_1	P	RATIO	χ^2_1	P
1-2	1.48	/	/	2.2	/	/
3	0.68	0.69	>0.05	0.23	9.96	<0.005
4	0.83	0.87	>0.05	0.32	9.66	<0.005
5	0.68	0.66	>0.05	0.16	13.09	<0.001
6	0.59	1.32	>0.05	0.29	9.24	<0.005
7	0.32	4.18	<0.05	0.31	11.04	<0.001
8	0.5	1.14	>0.05	0.16	10.88	<0.001
3-8	0.72	1.36	>0.05	0.25	13.45	<0.001

sheds with target-sheets (Spray:Target-Sheet = 0.23; $\chi^2_1=6.12$; $p<0.025$; $n=20$).

In contrast, the abundance of other phlebotomines from both treatments fluctuated non-significantly about the expected (Overall change, Spray:Control = 1.45 to 1.54; $\chi^2_1=0.06$; $p>0.05$; $n=10$; Target-Sheet:Control = 0.6297 to 0.74; $\chi^2_1=0.79$; $p>0.05$; $n=10$) (Fig. IV.2a). In consequence, the ratio of *Lu.longipalpis*:Other Phlebotomines in sprayed sheds also fell significantly after treatment (Overall change = 326 to 121; $\chi^2_1=9.88$; $p<0.005$; $n=10$), and was borderline-significant in sheds with target-sheets (Overall change = 244 to 148; $\chi^2_1=3.65$; $p\approx 0.05$; $n=10$).

Dining-huts. Catches in dining-huts were made from rounds 1-7 only, that is up to week 12 post-intervention. In homesteads with a target-sheet the abundance of *Lu.longipalpis* from dining-huts was significantly greater than expected on round 5 (week 7) (Fig. IV.2b, Table IV.4). However, there is no significant overall change in ratio after either treatment.

Similarly, there was no overall change from the expected abundance of other phlebotomines from either treatment (Target-Sheet:Control = 1.12 to 1.2; $\chi^2_1=0.03$; $p>0.05$; $n=12$; Spray:Control = 0.45 to 1.8; $\chi^2_1=2.33$; $p>0.05$; $n=10$).

Houses. Catches in houses were also only made from rounds 1-7. As for dining-huts, there was no significant deviation from the expected ratio (treatment:control) of *Lu.longipalpis* abundance (Fig. IV.2c, Table IV.5). In the homesteads with target-sheets, the ratio remained very close to the expected. In the homesteads with sprayed sheds, the ratio was consistently lower than expected, but the overall trend was not significant.

Table IV.4. Changes in the ln Odds Ratio (treatment:control) of *Lu.longipalpis* from the dining-huts of target and spray shed homesteads.

ROUND	TARGET (n=6)			SPRAY (n=5)		
	RATIO	χ^2_1	p	RATIO	χ^2_1	p
1-2	1.88	/	/	1.48	/	/
3	1.14	0.15	>0.05	0.68	0.16	>0.05
4	2.39	0.07	>0.05	2.56	0.28	>0.05
5	11.45	4.09	<0.05	2.87	0.55	>0.05
6	1.58	0.02	>0.05	2.55	0.16	>0.05
7	0.55	2.47	>0.05	1.2	0.04	>0.05
3-7	1.94	0.01	>0.05	1.8	0.02	>0.05

Table IV.5. Changes in the ln Odds Ratio (treatment:control) of *Lu.longipalpis* from the houses of target and spray shed homesteads.

ROUND	TARGET (n=10)			SPRAY (n=10)		
	RATIO	χ^2_1	p	RATIO	χ^2_1	p
1-2	0.82	/	/	2.6	/	/
3	1.03	0.21	>0.05	2.06	0.17	>0.05
4	0.86	0.01	>0.05	1.95	0.17	>0.05
5	1.32	1.61	>0.05	1.85	0.35	>0.05
6	0.85	0.01	>0.05	1.17	2.69	>0.05
7	0.68	0.09	>0.05	1.24	1.33	>0.05
3-7	1.04	1.63	>0.05	1.71	1.64	>0.05

Again, there was also no significant change from the expected abundance of other phlebotomines in either treatment (Overall Change, Target-Sheet:Control = 3.96 to 0.8; $\chi^2_1=3.33$; $p>0.05$; $n=10$; Spray:Control = 2.48 to 3.93; $\chi^2_1=3.14$; $p>0.05$; $n=10$).

Gonotrophic state. Dividing females in sheds by gonotrophic state, there is a tendency in both treatments for the abundance of gravid flies to be closer to the expected than either unfed, new-fed or old-fed flies (Fig. IV.3). However, the overall ratio of abundance of gravid females between treatment:control sheds is not significantly different from that of all other states combined (Gravid:Other Gonostates = 0.62 to 0.24; $\chi^2_1=2.79$, $p>0.05$, $n=40$).

Mortality rate. The difference between control and treated sheds in the proportion of flies found alive in traps is shown in Figure IV.4, setting the control to one.

No baseline data were collected for mortality, and for the analysis it was assumed that the proportion live in control sheds was the expected value in the treated sheds: in other words, barring the effect of the treatment, survivorship should be the same in treatment and control sheds. In support of this assumption, target removal on rounds 6 and 7 resulted in a significant increase in survivorship approximately equivalent to that in control sheds from the same rounds (-Sheet:+Sheet = 1.29 to 0.71; $\chi^2_1=4.14$; $p<0.05$; $n=24$) (Fig. IV.4, open squares).

Overall mortality was significantly greater in both treatments than in the control sheds (Table IV.6). However, by round 7 (week 12 post-treatment), the difference in both treatments ceased to be significant, and tended towards the expected, suggesting a reduction in the effect of the insecticide.

The overall mortality rate of other phlebotomines caught in treatment sheds is also significantly lower than controls (Target-Sheet:Control = 0.21 to 0.42; $\chi^2_1=5.04$; $p<0.05$;

Figure IV.3. Changes over time in the abundance of unfed (■), new fed (+), old fed (x) and gravid female *Lu.longipalpis* (Δ) caught in treated sheds, relative to control sheds (set to 1).

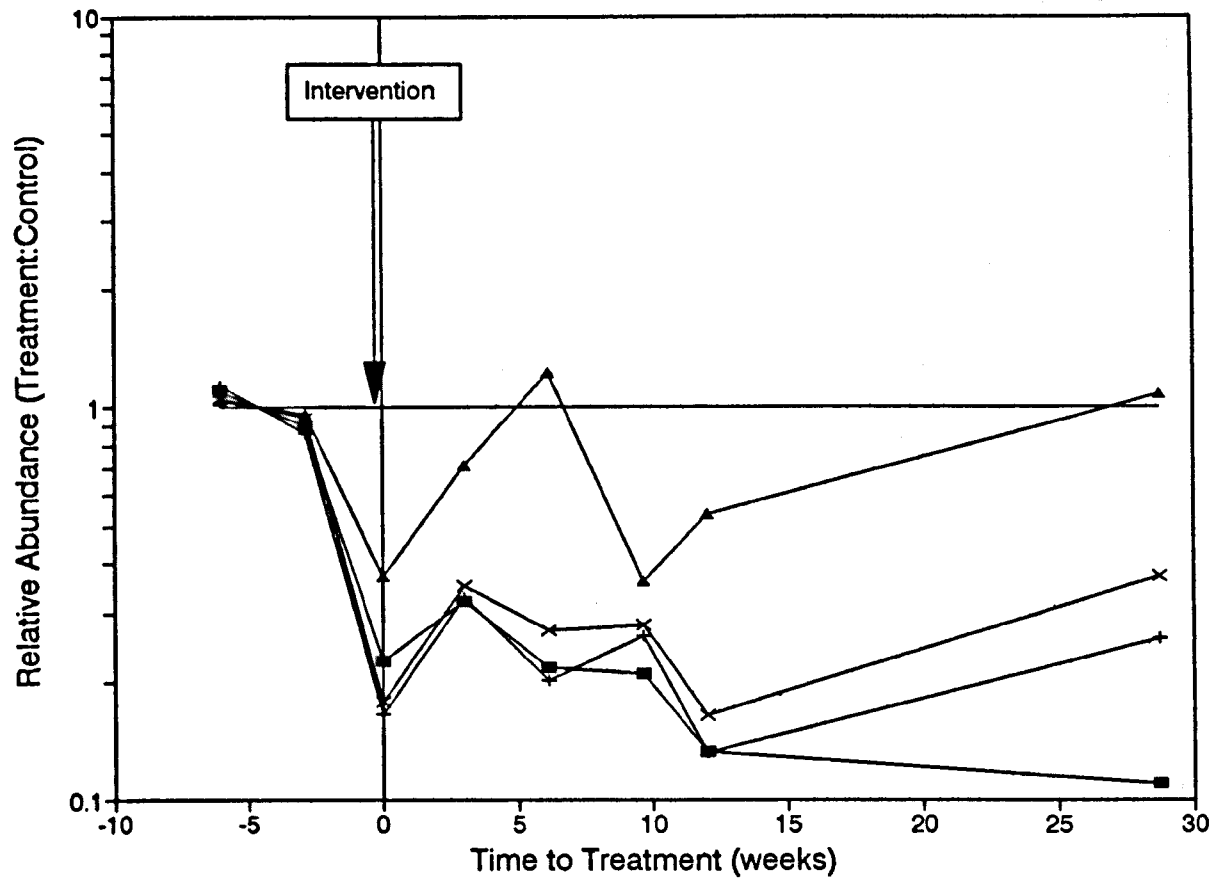


Figure IV.4. Changes over time in the ratio of live:dead *Lu.longipalpis* (solid line) and other phlebotomines (broken line) in sprayed sheds (Δ) and sheds with target-sheets (+), relative to control sheds (set to 1). Ratio of live:dead *Lu.longipalpis* in sheds with target-sheets relative to the same sheds with target-sheets removed (\square).

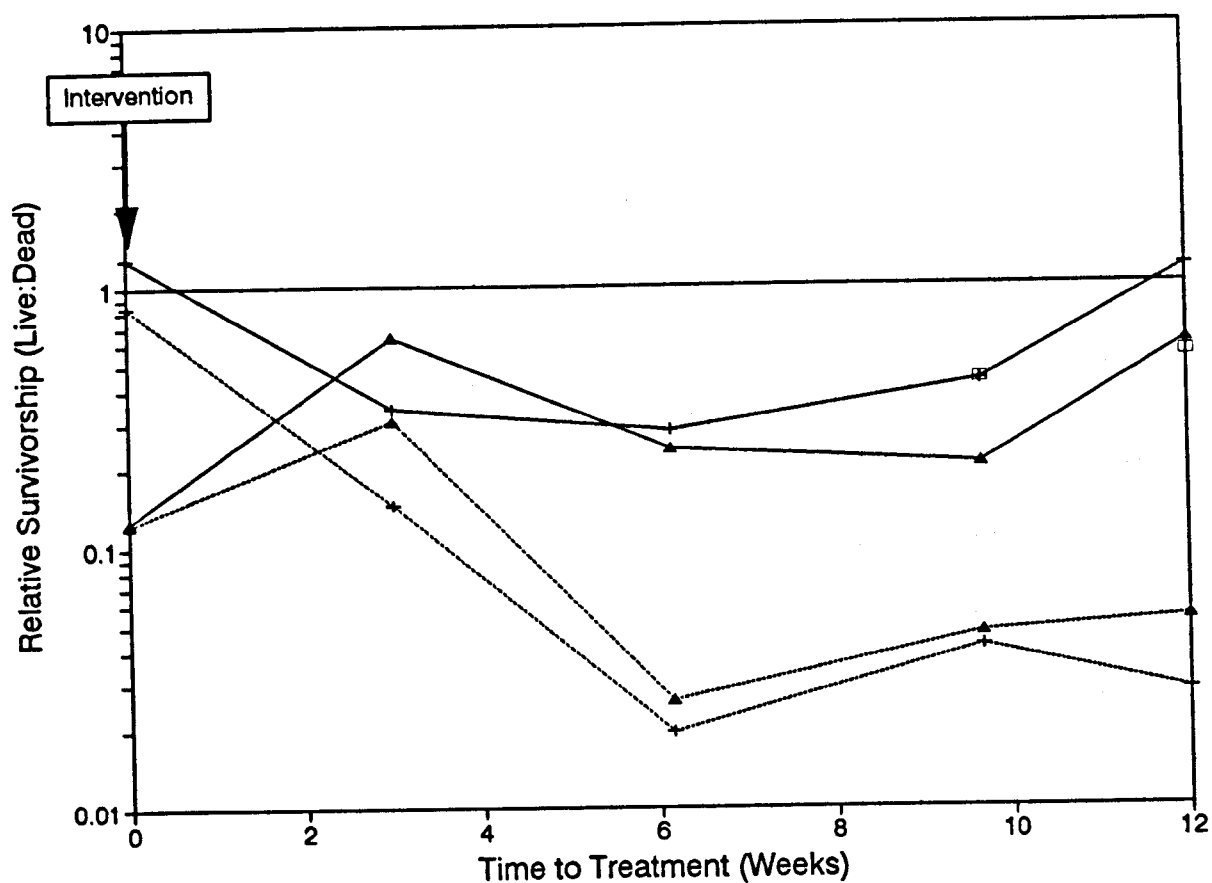


Table IV.6. Changes in the ln Odds Ratio of mortality (live:dead) of *Lu.longipalpis* in CDC traps from target and spray sheds.

	CONTROL (n=10)	TARGET (n=10)			SPRAY (n=10)		
ROUND	RATIO	RATIO	χ^2_1	p	RATIO	χ^2_1	p
3	0.13	0.16	0.62	>0.05	0.02	11.07	<0.001
4	0.17	0.06	7.89	<0.005	0.11	1.44	>0.05
5	0.34	0.09	7.6	<0.01	0.08	4.28	<0.05
6	1.59	0.68	5.71	<0.025	0.33	14.35	<0.001
7	0.61	0.71	0.06	>0.05	0.36	0.7	>0.05
3-7	0.4	0.18	11.53	<0.001	0.11	12.59	<0.001

n=20; Spray:Control = 0.25 to 0.42; $\chi^2_1=4.66$; $p<0.05$; n=20) (Fig. IV.4). Consequently, there is no significant overall difference in mortality between *Lu.longipalpis* and other phlebotomines in sheds with target-sheets (*Lu.longipalpis*:Other Phlebotomines = 0.4 to 0.21; $\chi^2_1=0.22$; $p>0.05$; n=10) or sprayed sheds (*Lu.longipalpis*:Other Phlebotomines = 0.11 to 0.25; $\chi^2_1=2.92$; $p>0.05$; n=10).

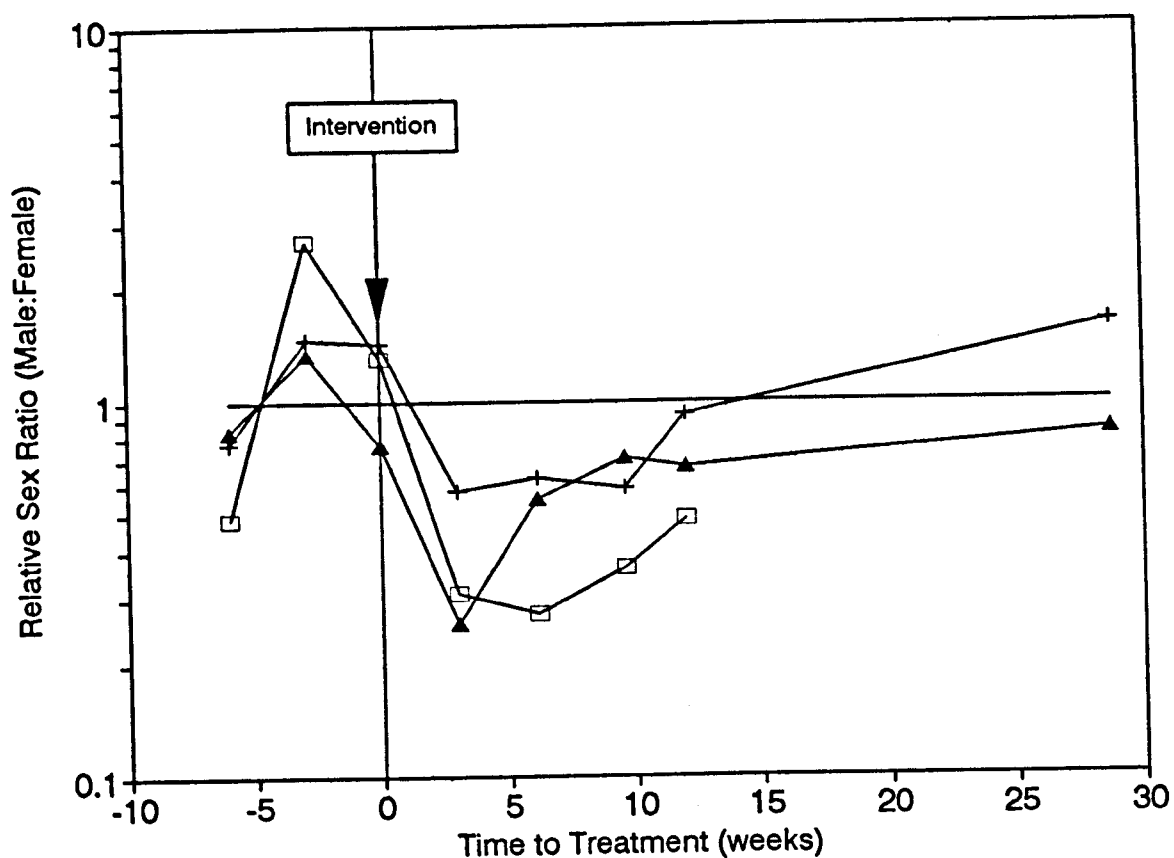
Sex ratio. There is a tendency for the sex ratio to become more male-biased than expected after both insecticide treatments, gradually returning to the expected over time (Fig. IV.5). However, it is not possible to test this in the normal way, since differences in sex ratio between the control and treatment sheds cannot themselves be modelled as a ratio. Nevertheless, a simplistic analysis can be made, comparing the mean sex ratio of control and treatment sheds before and after intervention.

The mean pre-treatment sex ratio in control sheds is 1.15 (male:female), not significantly different from that in sprayed sheds (Sex Ratio=1.18; $\chi^2_1=0.04$; $p>0.05$; n=10) or sheds with target-sheets (Sex Ratio=1.2; $\chi^2_1=0.07$; $p>0.05$; n=10). Post-treatment, the mean overall sex ratio in control sheds falls to 0.86, but the sex ratio in the sprayed sheds falls to 0.53, significantly lower than that of control sheds ($\chi^2_1=4.391$; $p<0.05$; n=10). Although the sex ratio in sheds with target-sheets also falls after treatment, the overall post-treatment ratio is only 0.81, not significantly different from the control ($\chi^2_1=0.04$; $p>0.05$; n=10).

Blanket Intervention

Pre-treatment data. Abundance of *Lu.longipalpis* in sheds, dining-huts and houses was 435.5, 42.8 and 45 respectively, very similar to the abundance in control homesteads from the

Figure IV.5. Changes over time in the sex ratio (males:females) of *Lu.longipalpis* in sprayed sheds (Δ), sheds with target-sheets (+) and VF sheds (\square), relative to control sheds (set to 1).



focal intervention. Other species of sandfly constituted less than 1% of the catch, of which, male *Lu.evandroi* (89%), *Lu.infraspinosa* (5%) and *Lu.evandroi* (1%) were most abundant, as in the focal intervention.

Chicken sheds. The reduction in *Lu.longipalpis* abundance as a result of spraying, though consistent up to round 7, is only approximately 45% that of the expected, considerably less than the focal intervention (Fig. IV.6a). Consequently, neither the overall ratio of treatment:control, nor any individual trapping round, was significantly different from expected (Table IV.7).

As for the focal intervention, abundance of other sandfly species showed no significant trend towards a lower than expected catch, and was in fact slightly higher than expected overall (Treatment:Control = 0.81 to 0.59; $\chi^2_1=0.59$, $p>0.05$, $n=6$).

Dining-huts. The abundance of *Lu.longipalpis* at dining-huts, unlike those in the focal intervention, showed a consistent and significant increase over that expected from rounds 4 onwards, averaging approximately 600% (Fig. IV.6b; Table IV.7). This was not repeated in the abundance of other sandfly species (Overall Change, Treatment:Control = 0.53 to 0.63; $\chi^2_1=0.25$, $p>0.05$, $n=5$).

Houses. There is no significant variation about the expected abundance of *Lu.longipalpis* in houses on any trapping round (Fig. IV.6c; Table IV.7). Similarly, there was no overall departure from the expected abundance of other phlebotomines (Treatment:Control = 0.65 to 0.76; $\chi^2_1=0.41$, $p>0.05$, $n=6$).

Figure IV.6. Changes over time in the abundance of *Lu.longipalpis* (solid line) and other phlebotomines (broken line) at VF homesteads (Δ), relative to control homesteads (set to 1).
Shed = a; dining-hut = b; house = c.

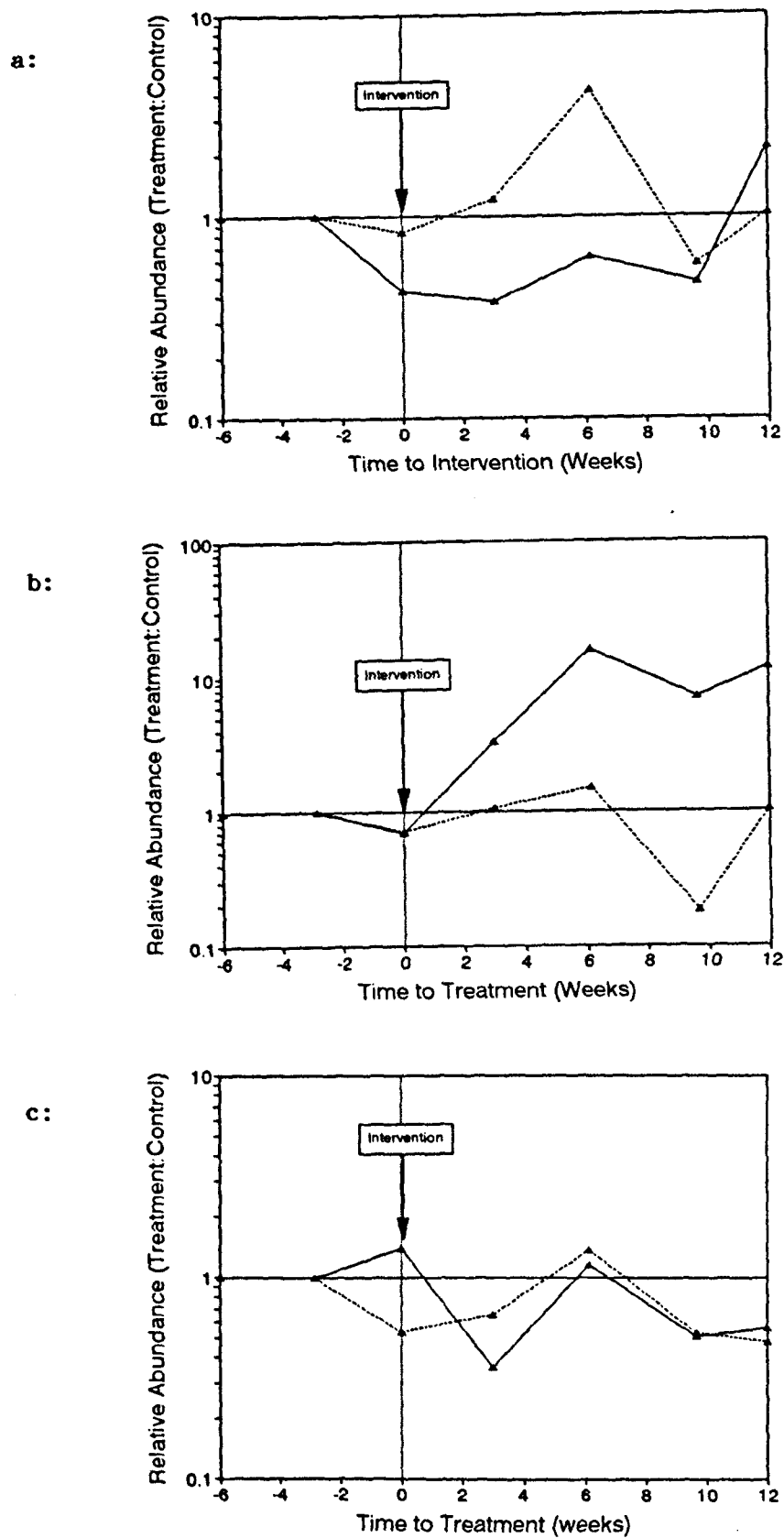


Table IV.7: Changes in the ln Odds Ratio (treatment:control) of *Lu.longipalpis* abundance in sheds from the blanket intervention (* p<0.05; ** p<0.01).

ROUND	SHED (n=6)		DINING-HUT (n=5)		HOUSE (n=6)	
	RATIO	χ^2_1	RATIO	χ^2_1	RATIO	χ^2_1
1-2	1.38	/	1.04	/	3.4	/
3	0.62	1.0	0.78	0.4	5.38	0.57
4	0.55	2.55	3.65	3.62*	1.3	0.95
5	0.92	1.43	17.28	6.88**	4.47	0.06
6	0.69	0.58	7.76	6.29**	1.97	0.28
7	1.15	0.06	12.86	4.85*	2.1	0.56
3-7	0.76	1.81	6.2	4.6*	2.55	0.13

Sex ratio. As was seen during the focal intervention, the sex ratio of flies in treated sheds during the blanket intervention became female-biased on round 4, and remained that way for as long as trapping continued (Fig. IV.5). Against the pre-treatment sex ratio in control sheds of 1.15 (male:female) that in treated sheds was 1.58, not significantly different ($\chi^2_1=1.2$, $p>0.05$, $n=6$). Post-treatment, male bias in the treated sheds (0.44) was significantly greater than that in the control sheds (0.86) ($\chi^2_1=4.88$, $p<0.05$, $n=6$).

DISCUSSION

Focal Intervention

Both spray and target-sheet treatments resulted in a large decrease in *Lu.longipalpis* abundance in sheds from the first night onwards (Fig. IV.2a, Table IV.3). We identify three possible explanations for this effect (not necessarily mutually exclusive): there is mass-killing of flies by the insecticide; flies are repelled by the insecticide; or attraction of flies to the treated site is reduced.

Insecticide-mediated killing of flies in sheds clearly occurred. Survival of *Lu.longipalpis* in both treatments was significantly lower than expected, and reversible in the target-sheet treatment (Fig. IV.4). An effect of similar magnitude was recorded for other *Lutzomyia* species, suggesting that similar levels of mortality were inflicted on all species.

However, despite the apparent equality of mortality rates between species in sheds, there was no reduction in the abundance of other phlebotomines to match that of *Lu.longipalpis* (Fig. IV.2a). A second factor must therefore be involved in the decrease in *Lu.longipalpis* abundance. Assuming that *Lu.longipalpis*, physically a relatively robust species, is not differentially sensitive to pyrethroids, and therefore that repellency was not a factor, two explanations for the disproportionate effect of insecticide on *Lu.longipalpis* abundance in sheds can be postulated.

The first explanation, proposed by Le Pont *et al* (1989) to explain their similar findings, is that *Lu.longipalpis*, unlike the other species caught, is disproportionately dependent on peridomestic maintenance hosts. Therefore the majority of *Lu.longipalpis*, but not other species, are forced to visit the treated sites and mass-killing results. In Le Pont's study the entire village was blanket sprayed, and most of the peridomestic maintenance host sites were treated. Our focal

treatment regime, in contrast, preserves large numbers of nearby hosts untreated, and we should not, therefore, expect mass-killing. Furthermore, mass-killing would not be instantaneous, yet in sprayed sheds fly abundance was immediately reduced by 90% (Fig. IV.2a).

The second explanation is that there is a disproportionate decrease in the attraction of *Lu.longipalpis* to treated sheds. Since host number did not vary, it is changes in pheromone production which are likely to have driven this effect, and pheromone has been shown to be more important than host kairomones in the attraction of *Lu.longipalpis* (Chapter III). Aggregation formation is primed by the disproportionately high number of pheromone-producing males which return to the site of the previous night's aggregation rather than distributing themselves randomly between all the available sites (Chapter III). By killing males before they can return on a subsequent night, treated sheds would be disadvantaged over neighbouring sites in terms of pheromone production and the attraction of flies to aggregations. The low numbers seen in sprayed sheds would therefore represent *de novo* generation of aggregations in the absence of a habituated male population returning night on night. Although nothing is known of the other species caught in our study, pheromone-mediated aggregation formation is uncommon in phlebotomine sandflies (Ward *et al* 1991.). If recruitment of other phlebotomines to the feeding site is therefore not dependent on the abundance of conspecifics, this might be sufficient to explain the lack of a decrease in their numbers. Put another way, disruption of pheromone-mediated attraction may be sufficient to explain the decrease in *Lu.longipalpis* abundance in treated sheds, without invoking mass-killing or repellency.

Evidence that abundance of male *Lu.longipalpis* in treated sheds is disproportionately reduced comes from the sex ratio, which becomes more female-biased than expected after insecticide application (Fig. IV.5). There is also direct evidence, though not statistically significant, of a reduction in pheromone-mediated attraction to treated sheds. Gravid flies are

known not to respond to pheromone (Ward *et al* 1990), and therefore if the changes in *Lu.longipalpis* abundance were the result of a reduced pheromone signal, abundance of gravids should be least affected. This is the observation illustrated in Figure IV.3.

Away from sheds, there is no evidence of a mass-killing effect of focal target or spray treatments at other sites in the homestead. There was no significant and consistent change in numbers caught in dining-huts or houses (Fig.s IV.2b & IV.2c). Although the abundance of *Lu.longipalpis* in houses at homesteads with target-sheets did show a downward trend (not significant), this may be a result of a depletion in fly numbers as a result of either fly mortality or the attraction of the fly population to untreated neighbouring homesteads.

Blanket Intervention

Whilst the results of the blanket intervention must be treated as preliminary because of the short-comings of the controls used, they support the conclusions from the focal intervention and point to some novel and potentially important consequences of attempts at blanket intervention with insecticide.

The effect of spraying on fly abundance in sheds was poor, averaging 45% of the expected compared with 10% at focally-controlled sheds, and only significant on round four (Fig. IV.6a, Table IV.7). However, low as this effect was, as with the focal regime there was even less apparent effect on other phlebotomines, prompting the same explanation: namely that the relative attractiveness of a site is reduced upon treatment.

The poor reduction in *Lu.longipalpis* numbers could be interpreted as high immigration of flies from other areas. However, lower immigration rates would be expected here than in the focally sprayed sites, where large populations of flies are maintained at sheds only a few meters

away. A more plausible explanation is therefore that these flies come from the local adult population and breeding sites. Flies are still being killed, leading to a female-biased sex ratio (Fig. IV.5), but because, unlike the focal regime, there are no major aggregation sites left untreated, there is less competition from alternative aggregation sites, and recruitment is better maintained.

Although all the major aggregation sites in the blanket intervention were treated, the reduction in their attractiveness appears to have given the remaining untreated aggregation sites an advantage in the competition to recruit flies.

One of the few readily accessible sites left unsprayed was the dining-hut, and the gonotrophic composition of catches at these aggregations are similar to that at sheds, suggesting that females there are feeding, rather than resting (Table IV.1). In contrast to focally-sprayed sites, abundance of *Lu.longipalpis* in dining-huts in the blanket intervention increased significantly after a lag of one trapping round after spraying (Fig. IV.6b, Table IV.7). This suggests that dining-huts are increasing in relative attractiveness, compared with sheds. In this scenario, the lag of one trapping round before numbers increase represents the period during which males become habituated to the new site. As with sheds, there is no equivalent change in the abundance of other phlebotomines relative to the expected, supporting the assumption that the change in *Lu.longipalpis* abundance is real, rather than the artefact of a poor control.

Abundance of *Lu.longipalpis* in houses, though on average lower than expected, did not fall significantly; equivocal evidence of overall diminution of fly abundance in the area (Fig. IV.6c, Table IV.7). The fact that numbers of flies in houses did not increase as for dining-huts, and that there was a high proportion of gravid females (Table IV.1), suggests that, at least in the case of well-constructed houses such as these, the flies found within are accidental visitors, rather than pheromone-mediated aggregations.

Implications for the Control of Lutzomyia longipalpis

Control of the *Lu. longipalpis* population without the house (i.e. mass-killing) is desirable to reduce disease transmission within both the canine parasite reservoir and the human population, particularly if significant transmission to humans is occurring at outdoor sites such as the dining-hut. However, the results suggest that by disrupting male pheromone production at the sprayed sites, the first effect of spraying is not mass-killing, but an increase in the relative attractiveness of unsprayed sites. This is not to say that, with considerable effort, mass-killing cannot be achieved. But if coverage is only partial, then there is a danger that flies will become increasingly attracted to unsprayed sites, and that the biting rate on dogs and humans will increase. The definitive experiment remains to be done, but the preliminary recommendations to come out of this study are that sandfly aggregation sites at non-susceptible hosts are better left untreated unless the time, money and organization is available to carry out a blanket spraying programme effectively.

Sheet targets, which might greatly simplify the intervention process, were disappointingly poor at controlling fly numbers compared with spraying. It is possible that the proportion of flies that were marked by sheets in Chapter III were higher than the contact rate achieved here, because of the attractiveness of the sugar marking solution. However, even had targets been as effective as spraying, the other reservations about fly control would remain.

One way of maintaining recruitment of flies to a treated site in the absence of males is through provision of a synthetic male pheromone bait. This might ideally be combined with sheet targets, prepared to high standards at a central site, to form a baited target in animal pens, increasing the contact rate with the target and avoiding displacement of flies to other host feeding sites.

CHAPTER V

THE EFFECT OF BLOODMEALS ON PARASITE BURDENS

IN *Lutzomyia longipalpis*

SUMMARY

- (1) A series of laboratory experiments were conducted to investigate the effect of bloodmeals from different species on *Leishmania chagasi* promastigote infections in the sandfly *Lutzomyia longipalpis*.
- (2) Feeding blood and promastigotes simultaneously, it was found that five days post-feed a significantly greater proportion of flies fed on fox blood, as opposed to chicken blood, were infected.
- (3) A similar and significant effect was found whether blood was first heat-inactivated or not, suggesting that the cause of differential parasite clearance was not a blood-borne immune factor, but probably digestion-mediated.
- (4) These results superficially support the uncontrolled observations of other workers, who conclude that nucleated blood is differentially deleterious to parasites in the sandfly gut by stimulating the production of a DNAase.
- (5) However, in our experimental system, fox blood was digested more slowly than chick blood. This allowed the analysis of the correlation between parasite abundance and degree of digestion. This shows that even in fox blood, which is not nucleated, the progress of digestion correlates significantly with parasite clearance.
- (6) It is suggested the observed differences in parasite burden between flies fed on chick and fox blood are a result of different rates of digestion, and that the underlying mechanism of digestion-mediated parasite killing, far from being limited to nucleated bloodmeals, may be a common feature of all bloodmeal digestion.
- (7) An alternative explanation is therefore offered for the field observation of parasite rates in *Phlebotomus papatasi* caught near turkey sheds in Israel, based on the rate at which sandflies encounter infective hosts.

INTRODUCTION

In the Jordan valley, *Leishmania tropica* is transmitted in a zoonotic cycle between the colony dwelling rodent *Psammomys obesus* and the sandfly *Phlebotomus papatasi*. Schlein *et al* (1982a,b), found that turkeys introduced into the region proved very attractive to the sandfly, and *P.papatasi* caught in their vicinity were found not to be infected, compared to a usual rate of 20-50% infection. This, they suggested, resulted from a lethal effect of turkey blood on parasites in the fly.

From work in the laboratory, Schlein *et al* (1983) reported a significant decrease in the proportion of *P.papatasi* infected with *L.tropica* when flies were fed on turkeys. The effect was two-fold: a turkey bloodmeal protected against future infection from a membrane feed of rabbit blood and promastigotes; and when taken after an infective membrane feed, turkey blood cleared the parasite burden of flies. The control in every case was a cohort of *P.papatasi* which took the infective bloodmeal only, without a previous or subsequent meal of turkey blood. However, such a control does not allow for the possibility that the effect of turkey bloodmeals may be reproduced with other types of blood.

In defence of this shortcoming in their experimental design, Schlein *et al* (1983) cite Adler and Theodor (1929, 1930) as demonstrating that a second blood meal, either human or rabbit, does not reduce the rate of infection of *Ph.papatasi* with *L.tropica*. However, no reference to these results could be found in the original papers. Furthermore, such an experiment does not compare the effects of human and rabbit blood directly with that of turkey blood.

The evidence presented in Schlein *et al* (1983) for the protection from future infection as a result of a previous turkey bloodmeal is also open to the same criticism that there is no control for other bloodmeal types: the mechanism is not necessarily specific to turkey blood, and

the control is not sufficient to prove that it is.

Schlein *et al* (1983) postulated that the mechanism of differential promastigote killing by turkey blood is elevated DNAase levels in the sandfly gut as a result of the nucleated turkey erythrocytes. They found, with what degree of significance they do not say, that tritiated thymidine-labelled DNA digested by *P.papatasi*, fed 24 hours earlier on a turkey, amounted to 54.2%, compared to 29.7% in flies similarly fed on rabbits. However, there was no attempt to show a direct correlation between DNAase activity and anti-parasitic activity in the gut.

Chickens also have nucleated erythrocytes, and they are of considerable importance as a maintenance host for *Lu.longipalpis* in our study area. In addition, evidence from Chapter II suggests that *Lu.longipalpis* will take more than one blood meal in a gonotrophic cycle, and that the likelihood of doing so increases with fly density. This behaviour, not previously thought to be typical of the species, is similar to that of *P. papatasi* (Schlein *et al* 1983) and would greatly increase the potential for a fly taking an avian bloodmeal and losing its infection. If the probability of this is also density-dependent, it may also have a regulatory effect on parasite transmission rates.

Despite the obvious short-comings of the work reported by Schlein and his co-workers, we were therefore led to address two questions relating to our own system - that is, with *Lu.longipalpis*, *L.chagasi* and chicken blood. Firstly, is chicken blood differentially lethal to established promastigote infections in the sandfly gut, and secondly, if so, is the effect mediated by digestive processes or other factors such as blood-borne immune components?

MATERIALS & METHODS

Sandfly Stocks

Lu. longipalpis were from a laboratory colony in the fourth, fifth and sixth generations, originally taken from Salvaterra district, Marajó Island. These were of the type whose males bear a single pale spot on abdominal segment IV (Ward *et al* 1988). Apparently only one member of the species complex is to be found on this part of Marajó Island (Dye *et al* 1991), although it is possible that more than one species exists with a single tergal spot.

Chick and Hamster Feeding

Golden hamsters (*Cricetus auratus*) and 1-2 day-old chicks were partially shaved, and restrained in small wire cages and placed in 20x20cm net cages containing a mixture of male and female sandflies. The cage was covered to exclude light and left overnight from 18.00-07.30hrs. Neither host had previously been exposed to sandflies.

Membrane Feeding

Rabbit and chick blood was taken by direct cardiac puncture. Rabbits had not previously been bitten by sandflies, and chicks were 1-2 days old. Blood from the crab-eating fox, *Cerdocyon thous*, was taken under anaesthetic from a wild-caught, IFAT-negative animal (Lainson *et al* 1990). All blood was taken on the same day, defibrinated with glass beads, stored at 5°C and used within 3 days. Heat inactivation, when carried out, was in a 56°C water bath for

20 minutes. This haemolysed the fox blood, but not the chick or rabbit blood.

We used a Haake FJ membrane feeder (U.S.A., N.J., 07662 Saddle Brook). This apparatus passed water at 32°C through a glass tube, at one end of which was a depression. To this depression was added approximately 0.5ml of the feeding material, over which a 1-2 day old chick skin was stretched and taped. The tube was then inverted and supported vertically in the entrance sleeve of a 20x20cm net feeding cage containing a mixture of male and female sandflies. The cage was covered to exclude light, and flies were left to feed from 18.00-07.30hrs.

Parasite Stocks

L.chagasi metacyclic promastigotes (M2682, from the collection of the Wellcome Parasitology Unit, Belém, from a child in Bahia State, North East Brazil), passaged and raised on blood agar slopes for 7 days before use. Supernatant from the blood agar tubes was pooled, and divided equally between the equal volumes of blood to be used in the feeding tubes. In this way, parasite densities were equal between the different bloodmeals in any one experiment, but could vary between experiments, depending on the amount of parasite in the culture tubes.

Breeding Conditions

Post-feed, flies were maintained in breeding tubes. These were clear plastic, approximately 2cm diameter, 5cm high, with a 0.5-1cm diameter hole cut in the lid which was covered with sandfly-proof netting. Within, a Watmans filter paper disc covered the base. An upright of dead leaf - brown but not far decayed - of the *imbauba* tree (the large-leafed variety, latin name not yet known), collected from an area of nearby primary forest, bisected the tube

almost to its hight. These had been shown to give improved fly survivorship up to the beginning of oviposition and during the two-day oviposition period, compared to flies in regular tubes, which used an upright of concertinaed filter paper (data not shown). Flies were transferred to individual tubes on the morning post-feed, and maintained in the dark, in airtight glass cabinets at room temperature and 100% relative humidity until needed again.

Sandfly Dissection

Live flies were knocked down in their individual breeding tubes by exposure for 5-10 minutes to approximately -5°C . These were then washed in physiological saline with a little detergent to remove excess setae, and transferred to pure physiological saline to remove the detergent. The gut was removed to another drop of saline, and the contents were examined at x400. The hind-, mid- and fore-gut was viewed and parasite abundance was scored on a log scale as zero, 1-10, 11-100 and greater than 100. The amount of bloodmeal remaining was also recorded as large (++), small (+) or absent (-).

Experiment 1

This experiment adapts the methodology of Schlein *et al* (1983), adding two control groups, to investigate the effect of a chicken bloodmeal on an established parasite infection in the fly. The initial infection was produced by membrane-feeding flies on defibrinated, heat-inactivated rabbit blood with parasites. Post oviposition (day seven), equal sized cohorts of flies were fed on live chick, live golden hamster, or remained in their breeding tubes and were not fed. Three days later, surviving flies in each group were dissected.

Experiment 2

The protocol for experiment 1 was modified in order to improve the rate of survival to dissection. This was achieved by feeding promastigotes and blood together, rather than in separate gonotrophic cycles. Under natural conditions, only amastigotes would be ingested with the bloodmeal. However, since the experimental system seeks to imitate the situation where a new bloodmeal acts upon an already established promastigote infection in the sandfly gut, this was considered a reasonable experimental compromise.

Two groups of flies were membrane-fed on defibrinated, heat-inactivated fox or chick blood plus parasites. After five days, when parasite burdens would be substantial under typical laboratory conditions (R. Lainson, pers. comm.), all surviving flies were dissected. Fox blood was chosen as the control because as a natural host it should have a minimum anti-parasite activity.

Experiment 3

Was conducted as for experiment 2, but using un-inactivated blood. Heat inactivation helps to maintain the blood in a sterile condition, but also denatures complement and the cellular immune system (Cruikshank *et al* 1975). Comparison of the effects of heat-inactivated and un-inactivated bloodmeals on parasite burden can therefore be used to investigate the mechanism of anti-parasitic activity. If un-inactivated blood, but not heat-inactivated blood is lethal to the parasite, then that would suggest that parasite death is blood-mediated rather than digestion-mediated as Schlein *et al* (1983) suggest.

Experiment 4

The effects of heat-inactivated and un-inactivated blood on the parasite was also investigated *in vitro*. Promastigotes were added to four aliquots of blood: heat-inactivated fox; heat-inactivated chick; *un*-inactivated fox; *un*-inactivated chick. All four were maintained at 32°C in a water bath. Two drops were taken from each tube at time intervals and transferred to blood agar slopes. After seven days the supernatants of the blood agar slopes were examined for parasites, when the presence of parasites gives an index of parasite survivorship in the blood preparations.

RESULTS

Experiment 1

The protocol of Schlein *et al* (1983) presented some practical problems; of 131 flies initially membrane-fed only 13 (10%) survived to dissection in the second gonotrophic cycle. In addition to poor survivorship, it proved difficult to gauge when digestion of the second meal was advanced, because meals were small (probably a result of physical obstruction by the parasites) whilst the parasite burden kept the abdomen distended. On dissection, it was found that bloodmeal digestion was not far advanced (Table V.1), and all three treatments (hamster-fed, chick-fed and unfed) showed 100% infection with much greater than 100 parasites. We saw no difference in the fate of gut parasites, with or without chick or hamster blood, and at best, therefore, we can only conclude that because little bloodmeal digestion had occurred, there is no evidence to contradict the theory that nucleated bloodmeal digestion is differentially lethal to the parasite.

Experiment 2

Due to the shorter duration of the experiment, survivorship was much improved. At the simplest level, looking at proportions infected for the two treatments (fox or chick blood) and ignoring the size of parasite burden, a significantly larger proportion of fox-fed flies were infected (Yate's Corrected Chi-Squared = 9.88, $p=0.0017$)(Table V.2). However, survivorship was significantly lower in chick-fed flies (O.R.=0.024, $p<0.001$)(Table V.3). A blood agar culture using the remainder of the blood from this experiment showed fungal contamination of

Table V.1. Experiment 1. Numbers of *Lu.longipalpis* with parasite burden and amount of bloodmeal remaining in the gut, with or without a second meal of chick or hamster blood. ++ = much blood remaining in the midgut; + = little blood remaining; - = no blood remaining. N/A = Not Applicable.

Treatment	Undigested Blood	Parasite Numbers:			
		0	1-10	11-100	>100
Chick-Fed	++	0	0	0	4
	+	0	0	0	0
	-	0	0	0	0
Hamster-Fed	++	0	0	0	2
	+	0	0	0	2
	-	0	0	0	0
Control	N/A	0	0	0	5

Table V.2. Experiment 2. Number of *Lu.longipalpis* with parasite burden and amount of bloodmeal remaining in the gut, for flies fed parasites in *un*-heat-inactivated fox or chick blood.

		Parasite Burden:				
		<hr/>				
	FOX:	0	1-10	11-100	>100	TOTAL
Undigested Blood	++	2	0	1	2	5
	+	11	2	2	2	17
	—	27	6	10	2	45
	TOTAL:	40	8	13	6	67
	CHICK:	0	1-10	11-100	>100	TOTAL
Undigested Blood	++	0	0	0	0	0
	+	1	0	0	0	1
	—	18	0	1*	0	19
	TOTAL:	19	0	1	0	20

*Parasites present but all dead.

Table V.3. Number (column percentage) of *Lu.longipalpis* surviving 5 days post-feed with parasites plus fox or chick blood.

	FOX	CHICK	TOTAL
LIVE	81 (95%)	23 (28%)	104
DEAD	5 (5%)	59 (72%)	64
TOTAL	86 (100%)	82 (100%)	168

the chick blood, which might, therefore, have been present in the blood of the feeding tubes.

A repeat of this experiment was made, but the promastigote challenge failed to infect any flies.

Experiment 3

Two repeats were successfully conducted. In the first repeat (Table V.4a), the proportion of fox-fed flies infected was significantly greater than chick-fed flies (Fisher's exact test, $p=0.024$). For the second repeat (Table V.4b) the difference is insignificant on it's own, though in the same direction (Yate's corrected Chi-squared=1.96, $p=0.162$), but when the two repeats are combined the overall result is significant (Mantel-Haenzel weighted odds ratio, $p=0.013$).

Fox blood appears to take longer to digest than chick blood in experiments 2 and 3 (Tables V.2, V.4a & V.4b). This allows analysis of the fox blood data for experiment 2, and the second repeat of experiment 3, for the effect of degree of digestion on parasite burden. The fox

Table V.4a. Experiment 3. Number of *Lu.longipalpis* with parasite burden and amount of bloodmeal remaining in the gut, for flies fed parasites in heat-inactivated fox or chick blood.

First repeat.

		Parasite Burden:				
		<hr/>				
	FOX:	0	1-10	11-100	>100	TOTAL
Undigested Blood	++	0	0	0	6	6
	+	0	0	0	5	5
	—	0	0	0	4	4
	TOTAL	0	0	0	15	15
		<hr/>				
	CHICK:	0	1-10	11-100	>100	TOTAL
Undigested Blood	++	0	0	0	0	0
	+	0	1	0	1	2
	—	10	2	4	18	34
	TOTAL	10	3	4	19	36
		<hr/>				

Table V.4b. Experiment 3. Number of *Lu.longipalpis* with parasite burden and amount of bloodmeal remaining in the gut, for flies fed parasites in heat-inactivated fox or chick blood.
Second repeat.

		Parasite Burden:				
		<hr/>				
	FOX:	0	1-10	11-100	>100	TOTAL
Undigested Blood	++	0	0	0	2	2
	+	0	2	0	3	5
	—	6	2	4	7	19
TOTAL		6	4	4	12	26
	CHICK:	0	1-10	11-100	>100	TOTAL
Undigested Blood	++	0	0	0	0	0
	+	0	0	0	0	0
	—	11	0	6	7	24
TOTAL		11	0	6	7	24

blood data from the first repeat of experiment 3 cannot be analysed in this manner because one of the column total equals to zero. Expected results were calculated for both data tables, taking the experiment effect into account, and then recalculated allowing for a possible interaction between parasite burden and blood digestion. The fall in deviance between the two models was close to significance (Chi-square = 11.77, df=6; $0.1 > p > 0.05$), and this was interpreted as supporting the trend, seen in the tables, for decreasing parasite burden with increasing digestion.

If it is accepted that parasite burdens decrease as digestion proceeds, then 2x2 tables ought to be compiled using just the data from flies with 100% digestion of the bloodmeal; the results are otherwise biased towards the chick-fed flies, in which digestion is further progressed in all cases. Treating the results in this way, significance is considerably reduced (Mantel-Haenzel weighted odds ratio, $p=0.058$).

Experiment 4

There is a marked difference in parasite density between heat-inactivated and *un*-inactivated blood, whether chick or fox (Table V.5), although live parasites are present, at some level, at all times, in all treatments. Parasites from the *un*-inactivated chick blood also seem to have grown better than those from the *un*-inactivated fox blood.

Table V.5. Experiment 4. Change in parasite number *in vitro*, in four classes of blood, over time. HF: heat-inactivated fox blood; HC: heat-inactivated chick blood; UF: un-heat-inactivated fox blood; UC: un-heat-inactivated chick blood.

TIME (hours:minutes)	Treatment:			
	HF	HC	UF	UC
0.0	+++	++++	+++	++
0.15	++++	++++	++	++
0.30	++++	++++	+	++
1.0	++++	++++	+	+++
2.0	++++	++++	+	+++
4.0	++++	++++	+	++
6.0	++++	++++	+	+
8.0	++++	++++	+	++
12.0	++++	++++	+	++
27.0	++++	++++	++	+++

+ = >0,<1 parasite (average per 5 fields [erythrocyte monolayer] x40)

++ = 1-10 parasites (")

+++ = 11-100 parasites (")

++++ = >>100 parasites (")

DISCUSSION

Taken at face value, the results of the comparison between fox and chick blood suggest that chick bloodmeals cause greater parasite mortality than fox blood (Tables V.2, V.4a & V.4b). In addition, the effect is present for both heat-inactivated and un-inactivated blood. Together, these results argue that the parasite killing is digestion-mediated rather than a result of blood-borne immune factors, and support the thesis of Schlein and co-workers that the presence of nucleated blood is the important factor. From the *in vitro* experiment (Table V.5), the lethal effect of un-inactivated fox blood appears greater than that of chick blood, and if anything would be expected to bias the experiment against the observed result.

There are two reservations to this interpretation. Firstly, it is possible that the result of experiment 2 was affected by fungal contamination, although this is not certain. Secondly and more importantly, however, the fox and chick bloods were clearly being digested at different rates in the sandfly gut.

The observation by Schlein *et al* (1983) that parasite burden decreased with degree of turkey bloodmeal digestion was central to their proposal that bloodmeal digestion was the mechanism of parasite killing. In the present study, such an analysis was only possible with the more slowly digested fox blood, which is not nucleated, but demonstrates the same phenomenon. Furthermore, reanalysing the data comparing only flies with fully-digested bloodmeals, the difference in the proportion infected between fox- and chick-fed flies becomes insignificant, suggesting that a quantitatively similar digestion-mediated process is occurring in both.

It therefore seems likely that this process is common to the digestion of many bloodmeal types, rather than just those with nucleated erythrocytes. Clearly, in order to demonstrate this conclusively more work is required. However, if these conclusions are proved correct, it suggests

that the effect of nucleated erythrocytes on the level of DNAase in the sandfly gut is irrelevant to parasite killing.

Subsequent work has concentrated on proteolytic trypsin- and chymotrypsin-like enzymes in *P. papatasi* (Schlein & Romano 1986, Borovsky & Schlein 1987). It was found that *L. major* promastigotes had a regulatory effect on levels of these enzymes in the sandfly gut, but that *L. donovani*, to which *P. papatasi* is normally refractory, did not. However, by adding soybean trypsin-inhibitor to the infective meal, *L. donovani* promastigotes were enabled to survive and multiply. The inference is that levels of these enzymes are important for the modulation of vector competence. There has been no work to date on variation in the production of these enzymes with different bloodmeals, but if blood from different animals were to have different effects, it might depend on the degree to which they stimulate production of these enzymes, rather than DNAase.

The second element of bloodmeal-induced parasite killing remains unresolved, namely whether a nucleated bloodmeal affords protection against future infection with parasites. The answer awaits future investigation, but if, as seems likely, DNAase levels are not the mechanism of parasite death, then new theories will be required. One possibility is that conditions of digestion in an 'experienced' gut are quite different from those during a first bloodmeal, and therefore possibly more lethal to the parasite (R. Lane, pers. comm.).

We are left without an explanation for the original field observations of Schlein *et al* (1982a,b), which identify a reduced rate of infection in flies around turkey sheds. A much simpler explanation than that proposed by Schlein *et al* (1983) is provided by basic epidemiological theory. Turkeys, like chickens in Brazil, are not susceptible to infection with *L. chagasi*. The local predominance of these animals as a reservoir host for the fly will therefore reduce the rate at which a fly takes an infected bloodmeal and therefore the proportion of flies

infected.

If future work suggests that bloodmeal digestion does indeed reduce parasite burdens in the vector, what is the significance of this process for parasite transmission?

On the one hand, it might increase the influence of the non-susceptible host population: as the proportion of hosts which are non-susceptible increases, infectious flies will not only stand less chance of biting a susceptible host; they will also stand less chance of remaining infectious until they do so.

On the other hand, flies with mature infections (which only take one gonotrophic cycle to mature in the lab) have great difficulty in taking a bloodmeal (Experiment 1, Schlein *et al* 1992). It therefore seems unlikely that a fly which only takes one meal per gonotrophic cycle would ingest sufficient blood to affect its parasite burden significantly. Flies which took several small meals, however, such as those in high density aggregations (Chapter II), might ingest parasites with the first meal and clear them with a non-infective meal on a subsequent night in the same gonotrophic cycle.

CHAPTER VI

GENERAL DISCUSSION

The Semiochemical Basis of Lutzomyia longipalpis Dynamics

In Chapter II, we observed a decrease in the rate of blood acquisition by female *Lu. longipalpis* with increasing female density at the host; a result of host-mediated interference. We argue that in the larger aggregations this cost is unlikely to be offset by improved quality of matings, and therefore does not conform to the Ideal Free Distribution. In failing to do so, female sandflies are misjudging the true value of aggregation sites for their lifetime fitness. This is principally a result of the male pheromone signal. There is an apparent lack of qualitative change in the sandfly response to pheromone: the immigration rate continues to rise and the emigration rate falls with increasing male abundance at all natural densities (Chapter III). If larger aggregations offer lower gains then this suggests that flies are not capable of making quantitative distinctions between aggregations of different sizes. The inverse relationship between immigration and emigration cues suggests further that the response to increasing pheromone is most easily explained as a diminishing likelihood of losing the odour plume.

In the sylvatic environment, where fly and host abundance are low and the risk of failing to mate and feed may be high, flies cannot afford to be choosy, and an unwavering assessment of the value of male pheromone may well be optimal. In the peridomestic arena, however, hosts are superabundant and males are distributed at a range of densities. Here, non-linear feeding gains (Chapter II) mean that the optimal strategy for a female is to select an aggregation of intermediate size, up to the point where the mating gains of increasing male abundance balance the feeding losses of increasing female abundance.

To achieve this, females would need to distinguish between aggregations of different sizes through the medium of pheromone concentration. That they cannot do so results in females placing an unduly high value on the largest aggregations: as a consequence, some sites are

overexploited and most sites are under-exploited. This difference between the perceived and true fitness value of host sites, and the consequent departure from IFD, has implications for vector control and disease epidemiology.

Consequences for Vector Control

The critical epidemiological measure of a fly's vectorial capacity is the infectious biting rate on susceptible hosts (Garrett-Jones 1964). Two key components are most readily influenced by insecticide intervention.

The first is mortality rate (mass-killing). This affects the number of bites an individual fly will take in a lifetime, and the chances of a fly surviving long enough to incubate the parasite and become infectious.

The second is the proportion of bites which a vector takes on hosts which are susceptible to infection (in the case of AVL these are canids and humans). As discussed in Chapter V, changes to the proportion of bites which are taken on susceptible hosts affects the rate of parasite transmission by altering the rate at which an individual fly bites infectious hosts (dogs) and acquires infection, and the rate at which that fly then encounters a susceptible host (dog or human) and transmits disease. If, as is suggested in Chapter V, there is also a risk of losing an infection every time a fly feeds, then the rate at which flies bite non-susceptible hosts may be even more important in reducing the transmission rate, since every meal that an infectious fly takes on a non-susceptible host is not only a lost opportunity for transmission on that occasion, but also during future bloodmeals.

Perceived through the antennae of a bloodthirsty female *Lu.longipalpis*, potential aggregation sites in the peridomestic world are apparent as plumes of host kairomones and male

pheromone. The location of these signals, and between sites their *relative* intensity, helps determine the probability of a female feeding on a particular host.

Traditional control methods targeting host sites attempt the finesse of maintaining the abundance of hosts apparent to the fly (because hosts at sprayed sites still produce kairomones), therefore achieving mass-killing without altering the proportion of bites taken on susceptible hosts.

For *Lu.longipalpis*, however, this strategy may fail if spraying disrupts the production of male pheromone, the most important of all signals (Chapter III). As a result, the aggregation site would be greatly obscured as the fly perceives it and the remaining sites rendered disproportionately attractive. However, since flies do not seem to be distributed according to IFD theory, but aggregated on only a subset of the available sites (Chapter II), spraying may cause the aggregations to be preferentially attracted to the unsprayed sites, with no resultant decrease in the abundance of hosts apparent to the fly, no reduction in feeding success, and therefore no mass-killing. Furthermore, these unsprayed sites are likely to be the hardest to control, such as dogs and outdoor humans. Thus, rather than achieving mass-killing, intervention may increase the proportion of susceptible hosts apparent to the fly population, and therefore the proportion of bites taken on susceptible hosts (Chapter IV).

There are three ways of dealing with the phenomenon of changing relative attractiveness of feeding sites through insecticide intervention.

Firstly, in aggressive pursuit of a mass-killing effect, blanket spraying may be attempted. The aim would be to maintain the relative attractiveness of host sites, and hence maintain recruitment, by reducing the attractiveness of all simultaneously. However, unless something approaching total coverage of potential aggregation sites is achieved, the problems outlined above may not be avoided.

A second, more sophisticated approach to control, still in pursuit of a mass-killing effect, would be to compensate for the fall in relative attractiveness of the sprayed sites by adding a bait. The most obvious choice of bait would be a synthetic form of the pheromone, and this is the goal of Prof. Richard Ward and co-workers at Keele University. The results of Chapter III suggest that were a synthetic pheromone available it might not only maintain recruitment but also increase it. However, this is not yet an option.

A third, more subtle approach to control eschews mass-killing, and takes advantage of our ability to dramatically affect the relative attractiveness of aggregation sites and alter the proportion of bites on susceptible hosts. Rather than spraying animal pens and therefore increasing the biting rate on susceptible hosts, an obvious solution is to treat susceptible host sites whilst leaving the animal pens untouched. The desired effect is a reduction in the proportion of susceptible hosts apparent to the fly population, and therefore the proportion of bites taken on susceptible hosts. There can be little doubt that a dramatic reduction in fly abundance can be achieved at individual sites, from the results of the focal intervention in Chapter IV. Since the aim is not mass killing, but a reduction in signalling male abundance on susceptible hosts, the more repellent the insecticide the better. Thus houses and dining-huts would be best treated with an excito-repellent insecticide such as DDT. Canids could be targeted by encouraging owners to let dogs sleep indoors, or the use of repellent dog collars.

Future Work

Vector Control. Clearly what is now needed is a large-scale intervention trial to test some of the theories advanced in the previous section. Ideally, such a trial would make a serious attempt at blanket spraying and mass-killing of the peridomestic *Lu.longipalpis* population.

Targets should include houses, dining-huts, animal pens and other permanent structures in the homestead. This should be compared with a minimalist approach providing 'personal protection' to the susceptible host population, by spraying human and canine habitations only. This might be reinforced with devices such as dog-collars impregnated with insecticide.

The important outcome measures would be infection rates in dogs and humans, and some independent measure of sandfly abundance. Note that neither sprayed or unsprayed permanent structures are likely to provide the latter. A possible solution would be to trap at caged hosts imported to the treated area for the trapping night only.

AVL Epidemiology. The present study suggests a number of lines of enquiry concerning the epidemiological consequences of density-dependent feeding success and the aggregated distribution of *Lu.longipalpis*.

We argue in Chapter II that density-dependent feeding success is mediated by host defensive behaviour. Defensive behaviour depends on the pain generated by biting flies. Work by Warburg *et al* (1994) has identified differences between sibling species of *Lu.longipalpis* in the levels of the erythema-producing peptide, maxadilan, in their saliva. Does higher maxadilan concentrations mean more host irritation, more defensive behaviour and therefore lower feeding gains? How would this affect vectorial capacity in different sibling species?

One way in which it might be raised by the suggestion that the mean volume of blood that a female obtains in a single gonotrophic cycle may be reduced as competition for the bloodmeal increases (Chapter II). If this is so, it implies a reduction in the probability of becoming infected with *L.chagasi*. A second way in which density-dependent feeding success may influence parasite transmission is raised in Chapter V: does repeated taking of small meals reduce the probability of an infected fly remaining so? The rate of parasite acquisition and loss by a fly are

obviously important components of vectorial capacity, and density-dependence in this process is worth investigating as a potential mechanism of parasite population regulation (Dietz 1988).

Naïve epidemiological models assume homogenous mixing between vectors and hosts. This is not so with *Lu.longipalpis* (Chapters II & III). It would be interesting to model the effects of aggregation on disease transmission. For example, is the prediction of IFD theory, that flies preferentially bite the least sensitive/most passive hosts, true in the field? In the case of susceptible hosts, does this result in a disproportionate number of bites on infected individuals and what are the implications for transmission and control?

Heterogeneous biting patterns also have implications for entomological monitoring, as mentioned above. The attractive properties of a site are not merely a function of host abundance (Chapter III), or likely to be independent of modifications to itself and neighbouring sites (Chapter IV). Careful thought must therefore go into the design of routine epidemiological monitoring and intervention measures.

Lu.longipalpis *Population Ecology*. Density-dependent feeding success will clearly have a regulatory effect on the sandfly population which is to a degree self-imposed by the sub-optimal distribution (Chapter II). Other density-dependent ecological factors limiting sandfly abundance are worth investigating, not least because of the potential effect on the outcome of control measures. Periods of density-dependent population regulation might be avoided when attempting to reduce population size.

Breeding site pressures are likely to be intense at certain times of the year. The fall in *Lu.longipalpis* abundance in control sheds from the onset of the rains (Chapter IV) suggests that this is a period of limiting breeding site resources. This is similar to the results of Zeledón *et al* (1984), and suggests that sandfly breeding sites in our study area are more commonly limited

by excess moisture, rather than insufficient moisture, which seems to be the case in Ceará, Brazil, and El Callejon, Colombia (Deane & Deane 1962, Morrison 1995). Although the major breeding sites of *Lu.longipalpis* remain undiscovered, there is scope for studies on density-dependent competition for larval resources. The effects on adult size - absolute size and its variance - of varying larval density on fixed resources have been studied for some mosquito species in the field (Fish 1985). Where larval *Lu.longipalpis* biology lies between the two extremes of scramble and contest (Nicholson 1954) can be readily investigated in the lab. Armed with this information, field catches can be made through the wet and dry seasons to study changes in larval population pressures with climate.

This study attempts to continue the work begun by Professor L.M. Deane and extended by Professors R. Lainson FRS, J.J. Shaw and R.D. Ward and Dr.s P. Ready, L. Ryan, C. Dye, C.R. Davies, R.J. Quinnell and many others who have investigated the field ecology of *Lutzomyia longipalpis*. The call for continuing research, above, covers only a fraction of what might still be done. What is clear, however, is that a detailed understanding of population dynamics, demanding rigorous field study, is essential for the effective design and implementation of control measures against *Lu.longipalpis* and American Visceral Leishmaniasis.

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